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INVESTIGATIONS ON THE CARRIER RATE OF PASTEURELLA MULTOCIDA IN BLACK RATS (RATTUS RATTUS) IN A COMMERCIAL QUAIL FARM

Mwankon, Esther S., Odugbo\*, M. O., Jwander, L. D., Olabode, V., Ekundayo, S O. Musa, U., Spencer, T. H., Isa , S. I., Kaikabo, A. and Simon Boss, S. National Veterinary Research Institute, Vom, Plateau State, Nigeria

\*Correspondence: odugm@yahoo.com; Phone: +234 803 5956 289

#### Abstract

The aim was to investigate the level of *Pasteurella multocida* infection from two anatomic sites of black rats (*Rattus rattus*), popularly referred to as house or roof rats in a commercial quail farmhouse with recurrent fowl cholera outbreaks and also to evaluate the association between the *P. multocida* found in rats co-habiting quail poultry houses and isolates from outbreaks of fowl cholera. Thus 100 pharyngeal and 100 rectum swabs samples taken from rats co-habiting farmhouse were obtained and evaluated bacteriologically for isolation of *P. multocida*; 54% of pharyngeal swabs and 62% of rectum swabs were positive for *P. multocida*. Extended phenotypic characterization of the isolates confirmed the presence of subspecies *P. multocida multocida septica* and *gallicida* were not encountered. Ramdom serotyping of 5 isolates each from the two sites confirmed serotypes A:4. Fowl cholera outbreaks were confirmed on the quail houses and carrier rats had the same *P. multocida* subspecies and serotype as the infected quail. The public health significance of the finding is also discussed.

#### INTRODUCTION

Bacteria included in the genus *Pasteurella* (family Pasteurellaceae) are commensals and occasional pathogens of many species of domestic and wild animals. Since first isolated by Pasteur as the causative agent of fowl cholera in 1880, the genus *Pasteurella* has

undergone many taxonomical and nomenclatural fluxes (1). Until recently, the genus included species formerly known as *Pasteurella pestis*, *P. pseudotuberculosis*, *P. enterocolotica*, *P. tularensis*, and *P. novicida*. The first three now constitute the genus *Yersinia* (family Enterobacteriaceae). The two remaining species, the tularemia agents, have been given the generic name *Francisella.* (2).

Nearly half a century after the first isolation of Pasteurella, bacteria with common biochemical and morphological features were grouped together Pasteurella multocida. as Diagnosis of pasteurellosis depends on clinical appearance, and results of culture on blood agar. Colonies are small, gravish and non-hemolytic. P. multocida are small non-motile, Gramnegative cocobacilli often exhibiting bipolar staining, oxidase- and indole-Ρ. multocida positive. strains are currently classified into 5 serogroups (A, B, D, E and F) based on capsular antigens and further classified into 16 serotypes based on lipopolysaccharide antigens Despite serological (1).similarities, *P. multocida* species is subdivided into three established subspecies: subsp. multocida subsp. septica and subsp. gallicida (3). A fourth subspecies (tigris) associated with tiger bites was recently proposed (1).

Many species of birds and mammals, including human beings, are susceptible to P. multocida infections. P. multocida has a global distribution and the organism is a normal flora of the upper respiratory and gastrointestinal tracts in a number of animal species. In cattle, sheep, goats, pigs, rabbits as well as domesticated and feral birds, Ρ. multocida causes а life-threatening pneumonia and septicemia. In humans (especially the immunocompromised) who come in contact with animals with P. multocida infection, septicemia and chronic abscesses characterized by extensive edema and fibrosis may result (4, 5).

cholera caused by Fowl specific serotypes of P. multocida is a cause of concern in poultry industry where it causes economic losses in the form of death, treatment cost and labor. Official registrations of clinical disease outbreaks underestimate the prevalence of fowl cholera in Nigeria, although available records indicate that the disease is a major constraint in the increased production of poultry

chickens, turkeys, ducks, geese and quail in Nigeria. Outbreaks may result in very high mortalities of up to 80% (6 – 9).

A major issue for control of P. multocida infection in poultry flocks is the tendency to have reservoir or long-term carriers that periodically shed bacteria to the environment and contributes to the spread of infection within flocks. Carriers are animals that after initial infection continue to have the infection internal organs either in and continuously or intermittently shed high numbers of the organism through feces, aerosol etc. Vermin are known to be a significant reservoir for *P. multocida* causing fowl cholera (4). In poultry houses the most studied reservoir of P. multocida is the common brown rat (Rattus norvegicus) found predominantly in cooler regions notably Europe and North America. The black rat found in tropical countries are much less studied With an estimated 10 million rat population in the world, Wincewicz (10) described rats as belonging to the most troublesome and detested plaques

tormenting people from the beginning of mankind. The economic damages inflicted on the human and animals by rats are mainly caused by their feeding routines and the serious hazard which results from the undisputed role in the epizootiology of many infectious and invasive diseases. The role of the brown rat in the epidemiology of fowl cholera in chicken and turkey farms is well documented (11, 12). This investigation reports the role of the black farm rat (Rattus rattus) which is largely confined to warmer areas of Asia and Africa, in the epidemiology of P. multocida infection in a commercial quail farm in north central Nigeria. The public health significance of the findings is also highlighted.

#### Materials and Methods

#### Rats

The black rats were obtained from the farmhouse by clubbing them to death. The killed rats were either processed immediately or kept in the deep freezer for not more than 3 days before processing. The rats were often found in dark gaps of building seats, in wall corners, and in warm cellars. It is omnivorous and active 24 hours a day. It feeds everywhere: at cereal store or poultry houses, in waste dumps and shop store houses. Generally, it feeds on people's and animals food as well as on plants and small vertebrates.

#### Farm

Over a 6-month period, rats inhabiting two rooms housing flocks of approximately 90,000 commercial Japanese quail (Coturnix coturnix *japoninica*) on a well-managed farm were chased and clubbed down by farm workers. The quail flocks were usually housed in cement buildings with concrete floors covered with wood shavings and the quail were reared on the floor with feeds and water provided in troughs. Over the years (1999 - 2005), episodic outbreaks of pasteurellosis have been reported in the flock and also at the time of collection of these rat carcasses.

#### **Phenotypic Examination**

The killed rat heads were skin-shaven and the snouts aseptically snipped transversely with forceps to expose the nasal cavity from which swab samples were collected from nasal turbinate and mucosa and plated onto blood agar (Oxoid) for overnight culture at 37°C. Each rat was equally swab-sampled from the inner rectal mucosa for similar isolation of bacteria. *Pasteurella*-like colonies on blood agar were selected for identification, subspeciation (3), and in some cases, serotyping (13).

#### Results

#### P. multocida isolation

Of the 100 rat carcasses investigated for both pharyngeal and rectal isolation of *P. multocida*, 54 of the pharyngeal samples were positive, and 62 for the rectal samples obtained (Table 1).

#### Subspeciation

Some physiological characteristics are employed to differentiate P. multocida isolates into the various subspecies. The ability of the isolates to ferment mannitol, dulcitol and sorbitol was used to differentiate the subspecies (Pasteurella multocida multocida, P. m. septica or P.m. gallicida). All the isolates were positive for mannitol and sorbitol but negative for dulcitol - a reaction typical of P. m. multocida

 Table 1: P. multocida isolation from two
 anatomic sites of farm rats (Rattus rattus)

Sample	No. of sample	No. positive (%)
source		
Nasal	100	54
Rectal	100	62

## Serotyping

Indirect capsular-typing of five randomly picked P. m. multocida isolates performed was by using the hyaluronidase decapsulation test (13), and somatic serotyping of the isolates was performed by using the technique of Heddleston et al. (14). These random isolates were identified as P.m.multocida serotype A:4

#### Discussion

*P. multocida* is difficult to eradicate partly because of the presence of wildlife reservoir or alternative reservoir. *P. multocida* infection in population of black rats in this study showed a relatively high (over 50%) level of carrier rate when compared to other similar investigations (11, 12). However there was little in variation in the results from nasal to rectal samples obtained in this investigation. P. multocida has been recovered from the oropharynx of wild Norway rats in Baltimore (USA) and from the nasal passages of wild rats obtained from poultry farms with pasteurellosis (12). These results and those of Curtis et al. (11) emphasize that nasal cultures alone to vield results that are apt underestimate the incidence of infection. The prevalence of P.multocida in the nares of rats ranged from 38 to 43% according to past surveys by other workers. In the assessment/evaluation of the sensitivity and specificity of techniques employed, Curtis et al (8) reported that 41% of samples taken proved positive after mouse inoculation, compared to 14% using media alone. Against this backdrop more positive samples may have been encountered if mouse inoculation had also employed been in this investigation.

Evidence of rat mortality due to pasteurellosis was not observed in our study and it has been reported by Manning et al. (12) that rats in contact with fowl cholera do not develop acute infection but may become carriers of *P. multocida*. Although reports on naturally-occurring clinical infections in rats are lacking, or have received limited scientific study, anecdotal evidence suggest that *P. multocida* had produced pathological evidence of haemorrhagic septicaemia in affected organs (12), and Roberts and Gregory (15) also reported an epidemic of ophthalmitis due to *P. multocida* in hooded Lister rats.

The subspecies and serotype found in the rats correlate with those that have frequently been isolated from outbreaks of fowl cholera in the quail flock (9), lending credence to the role of the rats in the epidemiology of the episodic outbreaks often reported in the quail farm. This hypothesis is further substantiated by the fact that the disease was more frequent during the wet season and the cold harmattan period when rats migrated into buildings for warmth.

From a public health perspective, *P*. *multocida* is being added to the list of infective agents that are potentially

transmitted after an animal contact. Although statistics in Nigeria is lacking, it is documented that P.multocida is estimated to infect 20% - 50% of the 1-2 million Americans (primarily children) who are bitten or scratched each year by a variety of animals including dogs, cats, pigs, rats, lions, opossums and rabbits (16). Just as it has been estimated that as many as 66% of dogs and 90% of cats are colonized with this organism, typically in the respiratory and gastrointestinal tracts, our study also depicts a high percentage carriage of this organism in both tracts of rats. Persons at risk for infection related to animal exposure include veterinarians, farmers, livestock handlers, pet owners and food handlers. Immunocompromised patients should be alerted about the potential risk related to poultry and laboratory small animal and pet animal contact, even when animals are in apparent good health.

The isolation from pharyngeal and rectal regions means that the organism could be transferred via aerosol and feces deposited on troughs containing food or water. This study verified that aside from *Rattus norvegicus* species of rat, *Rattus rattus* has been incriminated as carrier of *P.multocida* and the rodent poses a major concern at the present time because of the readily transmission of *Pasteurella* spp from them to poultry.

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# ASSESSMENT OF LEUKOCYTE ESTERASE DIPSTICK TEST IN DIAGNOSIS OF CHILDHOOD URINARY TRACT INFECTION

\*ADELEKE, S.I\*, \*ASANI, M.O. and \*\* NWOKEDI, E. E. \*BAYERO UNIVERSITY, DEPARTMENTS OF PAEDIATRICS, AND \*\*MICROBIOLOGY & PARASITOLOGY, KANO

CORRESPONDENCE: DR. S.I. ADELEKE. Dept. of Pediatrics, Aminu Kano Teaching Hospital, Kano. . E- mail: <u>adelekesolo@yahoo.com</u>

#### ABSTRACT

This is a prospective study of urinary tract infection in 65 children (38 males and 27 females, M: F ratio 1: 0.7). Urine samples were evaluated by culture, microscopy and leukocyte esterase dipstick test. Positive urine culture, with significant bacteriuria was found in 19(29.2%). Urine microscopy for leukocyturia identified positive urine culture in 6 of the 19 samples, giving a sensitivity of 43.1%. leukocyte esterase distick test correctly identified 14 of 19 urine samples with culture proven UTI (74% sensitivity). The positive and negative predictive values were 37.1% and 87.2% respectively.

The leukocyte esterase dipstick test was found to be better than leukocyturia in detecting UTI. in conclusion, leukocyte esterase is sensitive in detecting UTI, easy to perform, requires less time and does not need a highly trained personnel for the test.

Key words: Urinary Tract, leukocyte esterase, Infection.

#### INTRODUCTION

Urinary Tract Infection (UTI) is a common childhood infection, the presentation of which is often nonspecific in infant and young children. (1,2,3) et al. Feature such as dysuria, frequency and urgency which in adults are characteristics of UTI, are not characteristic features of early childhood. Prompt detection and treatment of urinary tract infection is especially important in children because the developing kidney is more prone than the adult kidney to formation of renal scars and later to development of chronic renal failure following delayed or improper treatment of the infection (4,5)

The general laboratory diagnosis of clinical UTI is usually confirmed by microscopy and culture of well collected and promptly processed sample of urine. There might be many challenges in collecting proper urine samples in children especially in the developing world. Reliable screening tests for UTI will facilitate early diagnosis and treatment of

patients with positive urine samples while the negative samples identified early will reduce unnecessary urinalysis.

Leukocyte esterase is an euzyme from neutrophil not normally found in urine and is a marker of pyuria (6). A dipstick is available that tests for their marker. This study was undertaken to evaluate the use of test strip leukocyte esterase as a rapid screening method in the diagnosis of urinary tract infection among children suspected of UTI in Kano.

#### PATIENTS AND METHODS

The subjects consisted of 65 consecutive children presenting with features suggestive of urinary infection among the patients attending the paediatric unit of Aminu Kano Teaching Hospital, Kano. Midstream urine specimens were collected into sterile containers by clean catch method in the older children, while collection in infants was by means of sterile urine bag attached to the perineum after cleaning with 1% chlorhexidine.

The urine samples were taken to the laboratory within one hour of collection and ware processed immediately. Five millimeters (5mm) of leopful of the samples were seeded (inoculated) on to blood agar (for total colony count) and Mac Conkey agar (for differential count). The culture media were incubated at 37°C for 18-24 hours. A

colony count of  $>10^5$  organisms /ml of voided urine was taken as significant. Ten millimeters of the urine was centrifuged for five minutes, the supernatant fluid was then decanted and the remaining contents were shaken and two drops ware placed on a slide covered with a cover slip and examined under light microscopy for leukocytes, using a high power objective. Significant leukocyturia was defined as  $> 10^5$  cells per high power field.

Screening urine for leukocyte esterase was done by using multistix 10 SG (Bayer) test strip according to the manufacturer's instruction. The performance of the leukocyte esterase in detecting or otherwise of UTI, was statistically expressed in terms of sensitivity. positive predictive and negative predictive values.

The Chi-square  $(x^2)$  test was used to compare the results of leukocyte esterase tests with significant. leukocyturia in patients with culture proven UTI. A P-value of < 0.5 was regarded as significant.

#### RESULTS

Sixty-five consecutive children (38 males and 27 females giving M: F ratio of 1: 0.7). They were aged 4 days to 12 years. Thirty three (50.8%) of the 65 were aged two years and below; 29

(76.3%) of the 38 males were uncircumcised. The commonest symptoms seen in these patients were fever; dysuria and abdominal pain.

Positive urine culture with significant bacteriuria was found in 19 samples (29.2%) Escherichia coli was the predominant organism isolated. (Table II), urine microscopy for leukocyturia was significant in 19 urine samples, giving a sensitivity of 43.1 percent; the specificity was 79.7 percent while the positive predictive value was 41.1 percent. The leukocyte esterase dipstick test was positive in 31 (47.6%) of the urine samples.

Table III shows the performance characteristic of leukocyte esterase dipstick test in detecting urinary tract infection. The positive and negative predictive values were 37.1% and 87.2 percent, respectively. False positive leukocyte esterase dipstick test was observed to be common among the females 9(37%) of 27 had a false positive leukocyte

Age (months)	Males	Females	Total	%
<1	3	0	3	4.6
1 -12	7	3	10	15.4.
13 - 24	15	5	20	30.8.
25 - 36	6	4	10	15.4.
37 - 48	0	2	2	3.0
49 - 60	2	1	3	4.6
>/ 61	5	12	17	26.2
Total	38	27	65	100.

#### TABLE I: AGE AND SEX DISTRIBUTION OF THE 65 CHILDREN STUDIED.

#### TABLE II: MICRO-ORGANISMS ISOLATED FROM THE 19 URINE SAMPLES.

Micro-organisms	No. of urine samples.	Percent of Total
Escherichia coli	11	57.9.
Klebsiella	4	21.1
Protens	2	11.0
Psendomomonas	2	11.0
	19.	100.

Leukocyte esterase Test	Colonies	i	Total
Result	>/ 10 <sup>5</sup> Im	< 10 <sup>5</sup> Im	
Positive	15	22	37
Negative	4	24	28
	19	46	65

#### Table III: LEUKOCYTE ESTERASE DIPSTICK TEST COMPARED TO URINE CULTURE.

esterase dipstick tests, compared to 25.6% among males. False positive leukocyte esterase dipstick test was higher in uncircumcised (82%) than the circumcised (19%).

The performance of leukocyte esterase dipstick test was compared to that of significant leukocyturia among patient with culture proven UTI. The results showed the leukocyte esterase dipstick test was positive in 15 (78.9) of the 19 with culture proven UTI in contrast to only 6 (31.5) of the 19 who had significant leukocyturia in association with culture proven UTI. This difference was significant ( $x^2 = 64$ , df= 1, p < 0.02). Leukocyte esterase dipstick therefore, test was better than leukocyturia in detecting UTI.

#### DISCUSSION

The sensitivity of leukocyte esterase dipstick test for detecting UTI with significant bacteriuria in symptomatic patients in this study was 79%. This finding is similar to those reported by others who used similar criteria for significant bacteriuria (Gold smith et al 1990; Perry et al 1982; Wanamanda et al 1999). The specificities ranging from 78 to 96 percent reported by Gold smith et al (7) and Perry et al (8) were higher than the 41.1 percent obtained in the study. It is also lower than the 59.4% in a similar study by Wammanda et al (9). The reason for the differences may be due to the number of patients studied. There were 65 patients in this study while the number of patients studied by the workers cited above except Wammanda et al (9), ranged from 800 to over one thousand patients.

The low positive predictive value of 37.1 percent and high negative value of 37.1 percent obtained in this study are similar to the studies done elsewhere. Wiggelinkhuzien et al (8) from South Africa reported figures of 42.3 percent and 98.2 percent, while Le Jeune et al (10), reported figures of 42.3 percent and 97.6 percent respectively, while Wammanda et al (9), in Zaria reported 36.6 percent and 86.4 percent. The high negative predictive value is essential requirement of leukocyte esterase dipstick on screening test for urinary tract infection. A negative predictive value is the likelihood that a subject with a negative test does not have the disease (UTI) tested for.

A false positive leukocyte esterase test was common in female patients than males this has been observed by earlier workers also. (6) It has however been speculated that this difference in false positive rates between the sexes could be due to increased contamination of urine samples in the females by vaginal secretions. The study has also shown that leukocyte dipstick test is more sensitive than significant leukocyturia in detecting culture proven urinary tract infection. Infection of the urinary tract remains a significant problem in most Paediatric populations. The dipstick test of leukocyte esterase is a reasonable and rapid screening test which does not require highly trained personnel for diagnosis of urinary tract infections.

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## **ORIGINAL ARTICLE**

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## THE PREVALENCE OF TRYPANOSOME INFECTION IN TRADE CATTLE, GOATS AND SHEEP SLAUGHTERED AT THE KADUNA ABATTOIR

EZEBUIRO<sup>1</sup>O.G.C., ABENGA<sup>2</sup>J.N. AND EKEJINDU<sup>3</sup>,G.O.C. Entomology/Parasitology Division<sup>1</sup>, Nigerian Institute for Trypanosomiasis Research, P.M.B. 2077, Kaduna.(okwudiriezebuiro @ yahoo.com). Pathology/Epidemiology/Statistics Division<sup>2</sup>, Nigerian Institute for Trypanosomiasis Research, P.M.B 2077, Kaduna and Department of Medical Microbiology/Parasitology, College of Health Sciences, Nnamdi Azikiwe University, Awka.

#### ABSTRACT

The prevalence of trypanosome infection in trade cattle, goats and sheep was investigated in slaughtered animals at the Kaduna Abattoir. Wet, thin, thick films, animal inoculation, haematocrit centrifugation technique and buffy coat methods were used to detect rypanosomes in the jugular blood of the animals. The packed cell volume (PCV) was also determined. A total of 300 cattle, 300 goats and 300 sheep were examined within five months (September, 1998 -January, 1999) and the prevalence rates in cattle, goats and sheep were found to be 5.00%, 4.67% and 3.33% respectively.

Mean PCV of infected cattle was 20.33% against uninfected cattle 35.08%. In goats, the PCV was 20.29%, uninfected goats 31.56%; while that of sheep was 19.40% and uninfected 32.85%. Trypanosoma vivax infection accounted for 60%, T. brucei 26.67% and T. congolense 13.33% in cattle. In goats, T. vivax infection accounted for 71.43%, T. brucei 21.43% and T. congolense 7.14%. Also T. vivax infection accounted for 70%, T. brucei 30% and T. congolense 0% in sheep. Sex did not significantly (P>0.05) affect infection rates. Although the prevalence rate of trypanosomiasis in cattle, goats and sheep appeared low compared with the previous works, natural trypanosomiasis remains economically importance in cattle, goats and sheep in Nigeria.

Key words: Animal Trypanosomiasis, Prevalence, Kaduna, abattoir.

#### INTRODUCTION

Animal Trypanosomiasis still constitute a major threat to food security in several parts of sub-Saharan Africa (1). It is estimated that not less than 46 million cattle are at risk of becoming infected by tsetse-transmitted trypanosomiasis (1). Animal trypanosomiasis has been known to cause not less than 3 million livestock deaths each year, 20% less in calving, 25% reduction in

milk yields, 50% reduction in livestock numbers reduces (2)and work efficiency of animals thus hindering production (1).African crop trypanosomiasis has also been known as a major factor in the depopulation

of many parts of Africa since the beginning of last century (3). Trypanosome species of major threat to cattle, sheep and goats include Trypanosoma vivax, T. congolense and T. *brucei brucei* (3,4). Due to the absence of surveillance, the exact prevalence situation of the disease in many part of Africa is not well known. This has led to break down in the control strategy which has contributed to the current upsurge in both human and animal trypanosomiasis in several parts of Africa today (5,6). In Nigeria, Animal Trypanosomiasis currently ravages several parts of agro ecological zones of the country (7,8).

Although small ruminants may not often show clinical sings of disease and it is assumed to be rarely affected under natural conditions that and trypanosomiasis of sheep and goats is not a serious problem (9), Several experimental studies have shown that small ruminants are fully susceptible to infection with pathogenic trypanosomes (10,11). In addition, infection in sheep and goats is frequently reported from field surveys (12,13,14) and the economic impact of trypanosomiasis on small ruminants is substantial (14).

In an attempt to facilitate treatment and/or control of trypanosomiasis, it is imperative that early diagnosis be made to ascertain the prevalence of the disease. This can only be possible through the use of reliable and sensitive diagnostic procedures. The epizootiology of the disease in Nigeria (15,16) and other parts of Africa (17) indicate increases in infection rates and losses resulting from naturally acquired infections despite decades of attempt at control.

In this work, the prevalence of trypanosome infection in cattle, goats and sheep at slaughter at Kaduna Central abattoir is reported using the clinical signs and parasitological diagnostic techniques.

# MATERIALS AND METHODS Animals

The study was conducted at the Kaduna abattoir. A total of 300 cattle, 300 goats and 300 sheep making a total of 900 animals were sampled and examined, during the 5 months (September, 1998 to January, 1999) period. 5mls of blood was collected at slaughter from the cattle, goats and sheep into ethylene tetra-acetic acid (EDTA) bottle. Each sample was kept cool by placing in a box containing ice packs immediately after collection and transported to the laboratory for examinations.

#### **Clinical Diagnosis**

The animals were examined physically for manifestation of clinical symptoms. The history of animals was also taken to ascertain their source and passage through tsetse fly belts and general husbandry practice. Most of the animals were brought from the more Northern parts of Nigeria namely: - Sokoto, Kano, Katsina, Jigawa, Adamawa, Borno, Zamfara and Niger States.

#### **Parasitological Diagnosis**

The 5ml of blood collected in the EDTA bottle were subjected to diagnostic techniques of the Standard Trypanosome detection methods (STDM, 30) i.e. wet film (WF), Thin film (TF), Thick film (THF), Animal inoculation (AI) and concentration techniques (1) namely, Haematocrit Centrifugation Techniques (HCT,31) and Buffy Coat Method (BCM; 24).

#### **Differential Morphology**

Any organism with a free flagellum, very well developed undulating membrane and a small sub-terminal kinetoplast was classified as T. brucei, while an organism with a medium sized marginal kinetoplast but without a free flagellum and inconspicuous undulating membrane was identified as T. vivax (8). Also for T.congolense are absence of free flagellum, inconspicuous undulating membrane, and kinetoplast marginal and subterminal.

#### Packed Cell Volume (PCV)

The packed cell volume was determined for all blood samples as а haematological index. This was carried out just like haematocrit centrifugation technique and after spinning for 5 minutes, the length of the columns of the fluid plus cells, can be taken as direct measurements of the relative amount of the solid and fluid portions of the sample since the diameter of the bore is constant. The column of packed red cells is stated as percentage of the whole and it expresses the proportion of red cells. It is used as a quantitative expression of anaemia, a most useful index in assessing the progress of trypanosomiasis.

## RESULTS

Details of the prevalence of trypanosomes in cattle, goats and sheep are shown in tables I-v. The overall infection rate revealed that 5.00% of cattle, 4.67% of goats and 3.33% of sheep sampled were infected (table iv).

Trypanosome infection rates in the different sexes show that 8.43% of male and 3.69% of female cattle, 3.66% male and 5.05% of female goats as well as 3.39% of male and 3.32% of ewes sampled were infected (table vi). The differences in the mean packed cell

volume (PCV%) of trypanosome infected and those of non-infected animals are also shown on table vii. The volume mean packed cell of tryponosome infected was 20.33+3.31 while those not infected was 35.08+4.61. In goats, the mean packed cell volume of infected animals was 20.29+2.40 and that of uninfected animals was 31.65+6.21. the packed cell volume of trypanosome infected sheep was 19.40+2.59 as against 32.85+3.61 of uninfected sheep. The above drop in the mean PCV of infected animals differed significantly from those of uninfected animals (P<u><</u>0.05).

TABLE	I:	TRYPANOSOME	INFECTION	RATES	IN	DIFFERENT	BREEDS	OF	CATTLE
SLAUG	ΗT	ERED AT KADUN	A ABATTOIR	1					

Breed	No. Of Animal	No. of Animal +ve	T.vivax	T. congolense	T.brucei
White Fulani	238	11	2	8	1
Red Bororo	28	-	-	-	-
	10		1	1	
Adamawa Gudali	13	2	1	1	-
Ketekou	21	2	1	-	1
Total	300	15	4	9	2

# TABLE II: TRYPANOSOME INFECTION RATES IN DIFFERENT BREEDS OF GOATS SLAUGHTERED AT KADUNA ABATTOIR

Breed	No. Of Animal	No. of Animal	Т.	T. congolense	T.brucei
		+ ve	vivax		
Red Sokoto	200	10	6	1	3
West African Dwarf	72	4	4	-	-
Kano Brown	28	-	-	-	-
Total	300	14	10	1	3

# TABLE III: TRYPANOSOME INFECTION RATES IN DIFFERENT BREEDS OF SHEEP SLAUGHTERED AT KADUNA ABATTOIR

Breed	No. Of Animal	No. of Animal	Т.	T. congolense	T.brucei
		+ ve	vivax	-	
Yankasa	220	7	5	-	2
West African Dwarf	80	3	2	-	1
Total	300	10	7	-	3

# TABLE IV: OVERALL ANIMAL SAMPLED BOTH INFECTED AND UNINFECTED SLAUGHTERED AT KADUNA ABATTOIR

Animal	Total Nos.	Infected	Uninfected	Percentage infected (%)	Percentage Uninfected (%)
Cattle	300	15	285	5.00	95.00
Goats	300	14	286	4.67	95.33
Sheep	300	10	290	3.33	96.67

# TABLE V: OVERALL PERCENTAGE (%) INFECTION RATE OF DIFFERENT ANIMALS SLAUGHTERED AT KADUNA ABATTOIR

Trypanosomes	Cattle (%) (15)	Goats (%)% (14)	Sheep (%) (10)
T. vivax	60.00	71.43	70.00
T. brucei	26.67	21.43	30.00
T. congolense	13.33	7.14	0.0
TOTAL	100(15)	100(14)	100(10)

# TABLE VI: SEX DIFFERENCES IN TRYPANOSOME PREVALENCE PERCENTAGE OF ANIMALS SLAUGHTERED AT KADUNA ABATTOIR

Animal	Sex	No. of Samples	No. of Positive	Percentage Positive
		Examined	Samples	(%)
Cattle	Male	83	7	8.43
	Female	217	8	3.69
			_	
Goats	Male	82	3	3.66
	Female	218	11	5.05
Sheep	Male	59	2	3.39
	Female	241	8	3.32

# TABLE VII: MEAN PACKED CELL VOLUME OF DIFFERENT ANIMALS SLAUGHTERED AT KADUNA ABATTOIR

Animal	TRYPANOSOME Infected (PCV %)	Uninfected (PCV%) Mean ± S.E.
	Mean± S.E	
Cattle	20.33±3.31	35.08±4.61
Goats	20.29±2.40	31.65±6.21
Sheep	19.40±2.59	32.85±3.61

#### DISCUSSION

The trypanosome prevalence rate in different breed of animals for the past few years in ruminant ranged from 8.4% 15.53% (5,18,19,20). to Our findings of low overall infection rate in cattle, goats and sheep suggest that difference prevalence in of trypanosomes among ruminants may be as a result of the chemotherapeutic and chemoprophylatic campaigns of Governments or herd owners.

Since the majority of the animals sampled were brought from Northern part of Nigeria, its shows that the risk of bovine trypanosomiasis still exists, and the nomadic nature of husbandry practice is an important factor in the maintenance of transmission cycles of the disease (18).

Out of the cattle sampled in Kano, 7.6% were positive (19), out of 39 goats blood samples examined at Ilorin abattoir, only two were found with trypanosome infection (21). On the other hand, out of 58 goats blood samples examined at Nsukka, eight (13.9%) were positive for trypanosome

infection (22,23). At the Jos abattoir, out of a total of 960 goats screened, a 5% infection was found (23). This suggest that trypanosome infection rate in animals differ from one geoepidemiological zone to the other.

From the number of male animals sampled, this probably indicates that does influence their sex not susceptibility to the infection (9). The combination of parasitological techniques employed reduced the chances of missed diagnosis (15, 24) even though few cases of false negatives by microscopic examination were later confirmed to be positive through mice inoculation. Every parasitological diagnostic technique is an important tool in the epidemiological study of trypanosomiasis. However, their sensitivity cannot be compared with modern techniques such as ELISA (25,26-31). However, the combination of both techniques are necessary for improvement in trypanosomiasis surveillance which is essential in the management of control strategy. The findings in this study suggests that animal trypanosomiasis still constitutes a major threat to livestock and meat quality in Nigeria. Sustained surveillance of trypanosomiasis in cattle, goats and sheep is an important prerequisite for the enhancement of livestock production in Nigeria. The present effort to expand the animal industry in the country requires the knowledge of the disease problems that could be prevented and controlled. It will therefore be beneficial if the incidence and prevalence of trypanosomiasis in cattle, goats and sheep is investigated periodically.

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## **ORIGINAL ARTICLE**

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## QUANTITATIVE CHANGES IN ANTIBODIES AGAINST ONCHOCERCAL NATIVE ANTIGENS TWO MONTHS POST-IVERMECTIN TREATMENT OF ONCHOCERCIASIS PATIENTS.

Osue, H. O. \*, Galadima<sup>+</sup>, M., Engelbrecht<sup>Ψ</sup>, F., Odama<sup>Ω</sup>, L. and Edeghere<sup>#</sup>, H.I. \*Human Trypanosomiasis Research Division, Nigerian Institute for Trypanosomiasis Research (N.I.T.R.), Kaduna, Nigeria. &Microbiology Department, Faculty of Science, Federal University of Technology, Minna, Niger State, Nigeria. <sup>Ω</sup>National Institute for Pharmaceutical Research and Development (NIPRD), Abuja, Nigeria. <sup>Ψ</sup>Institut, abt. Parasitologie, Unversity of Heldelberg, Germany and <sup>#</sup>National Onchocerciasis Programme (NOCP), 1 Racecourse Road, Kaduna, Nigeria.

\*Correspondence: Hudu O. Osue, Human Trypanosomiasis Research Division, Nigerian Institute for Trypanosomiasis Research (N.I.T.R.), P.M.B. 2077, Surame Road, Ungwan Rimi GRA, Kaduna, Kaduna State, Postal code 80001, Nigeria. E-mail: <u>osueho@yahoo.com</u>

Running Title: Quantitative changes in antibodies.

#### Abstract:

Serum antibodies to Onchocerca volvulus native sodium duodecylsulphate slat extracted antigens and epitopes recognized by three monoclonal antibodies designated Cam8, Cam22, and Cam28 were measured using indirect (sandwich) and competitive enzyme-linked immunosorbent assay (ELISA). Paired serum samples (n=32) were obtained before and two months post-ivermectin treatment. Those with increases of ten percent and above ( $\geq 10\%$ ) were 16 (50%) for IgG, 13 (40.7%) for both IgG1 and IgG4. Nine (28.2%) for IgM, eight (25%) for IgG3, IgA with four (12.5%) was the least, while IgG2 was not assayed due to cross-reaction. The higher increases in IgG, IgG1 and IgG4 antibodies in females (n=16) than males (n=16) were significant by T-test of unpaired data (P<0.05). Those without onchocercal skin disease, OSD (n=18) had a significant increase of 20.5±29.6%, with pre- and posttreatment values of 0.59±0.15 versus 0.68±0.13 for IgG antibody (P<0.05). Both IgG1 and IgG4 antibodies for those with OSD (n=14) increased by 16.0±24.8%. Only IgG4 antibody increased with the presence of palpable nodule and higher skin microfilarial density. Trend exhibited by Cam 22 and Cam 8 were similar to that of IgG and IgG4, respectively. In conclusion, while IgG1 and IgG4 were both associated with skin diseases, IgG4 assay proved more suitable for onchocerciasis drug screening.

Keywords: Onchocerciasis, ivermectin treatment, antibodies, and antigens.

#### **INTRODUCTION**

Onchocerciasis or river blindness is one of the main causes of preventable blindness sub-Sahara in Africa. including Nigeria. Ivermectin® or Mectizan™ (a microfilaricide) is currently used for mass treatment of people in endemic areas. This strategy is preferred to larvicides and adulticides formerly used for fly control, which

have been abandoned because of the negative impact on environment. Infections were known to present with diverse clinical manifestations, even within the same organ. The main trust of immunology is to identify immune responses involved in immunopathology and/or immunopathogenesis of the disease, the molecules (immunogens) that can induce protective immunity for vaccine production, and antigens useful for immunodiagnosis. has been It suggested that it could serve as tool for drug screening and monitoring the efficacy of treatment (1).In onchocerciasis, humoral and cellmediated immune (CMI) responses are widely reported to vary from one person to another (2). Hence, the possible etiologic role particularly of parasitespecific antibodies has remained poorly understood. Understanding changes in immune responses after treatment in clinically defined patients could explain its secondary effects on pathology.

Changes in polyclonal, and parasite specific antibodies, proliferative T lymphocyte, and cytokine production have been documented after ivermectin treatment (3, 4, & 5). It has been postulated that immunologically mediated destruction of microfilariae could contribute to the pathogenesis of the disease. Dying parasites initiate local inflammatory reactions, with the result of "bystander" tissue damage, which cumulatively determines host pathology (6 &7). Absence of animal model precludes immunological studies; patients' responses to therapy and risk factors for clinico-pathological changes in human infections are evaluated (8). However, Steel <u>et al</u>. (5) had shown that previously recognized and unrecognized parasite antigens were released into circulation.

We report on the effect of ivermectin® on parasite antigen-specific serum antibodies two months post-initial treatment of individuals. This type of study is useful for indirect quantitative assessment of B-lymphocyte anergy (or tolerance) to parasite antigens and to show if changes in antibodies were associated with gender, age, host parasite burden, and/or pathological sequel.

# Materials and methods Sample population

After explaining to the participant in their dialect through an interpreter from the Local Government Area Health Department, full consent of individuals were obtained before been enlisted for

this study. Serum samples were collected randomly from individuals (n=32) comprising 13 males and 19 females that volunteered before initial ivermectin treatment was administered. Paired sera were obtained 2 months post-treatment. The subjects varied in age with a Sample population mean and standard deviation (SD) of 39.9±15.2 and ranges between 15-69 years. Sixteen of them had palpable nodule and the microfilarial density per skin snip was 26.05±35.13 with a range of 0-141. Among them, those with at least one form of skin clinical signs were n=14 and those without were n=18.

Antigens Detail method of preparing sodium duodecyl sulphate (SDS) extracted crude antigen has been described (9). The extract was supplied to the immunology Research Laboratory, NITR, Kaduna as part of a collaborative study Dr F. by Engelbrecht, Heldelberge University, Germany then a Visiting Scientist.

#### Monoclonal antibodies:

Three monoclonal antibodies (mAbs) designated Cam 8, Cam 22 and Cam 28 raised in mice were prepared by Engelbrecht *et al.*, (9). Only Cam 28 has been characterized and found sensitive to periodate. It reacts with 120 KD molecular weight antigens. These mAbs were provided by Dr F. Engelbrecht.

#### SEROLOGY

Serum samples were analyzed same day under similar assay conditions. Reactivity of serum antibodies with antigens and the three mAbs were tested with indirect and inhibition (competitive) ELISA, respectively.

#### Indirect sandwich ELISA

Immunoglobulin (Ig), IgA, IgM, IgG class and IgG1, IgG3 and IgG4 isotypes antibodies were measured using a modified protocol described by Engelbrecht <u>et al</u>., (9). Briefly, microtitre plates were coated with antigens diluted in carbonate/bicarbonate buffer (pH9.6) at 1:100, and incubated overnight at 4°C. All other steps were performed at room temperature (RT°C). The unspecific site were "blocked" with 200 µl per well of 1-2% bovine serum albumin (BSA) for 1 hour. Serum was added at 1:80, 1:200, and 1:160 for IgA, IgG, and IgM reactivity to SDS extract. Horseradish peroxidase conjugated to rabbit immunoglobulin anti-human IgG (Dako, Denmark) (code P214); IgM (P215) and IgA (P216) were applied at 1:1000, 1:400, and 1:500 diluted.

For isotypes assays serum was added at 1:500 for IgG1, 1:100 for IgG3 and 1:200 for IgG4. Thereafter, monoclonal antibody obtained from Sigma specific for each isotype, IgG1 (clone HP 6001, 1:2000), IgG3 (HP 6050, 1:8000) and IgG4 (HP 6025, 1:8000) were added at 150µl per well. It was followed by goat antimouse IgG (H+L) horseradish peroxidase conjugate (BIORAD) at 1:1000 dilution.

Antigen and antibody reactions were detected by addition of freshly prepared substrate solution containing 200µl orthophenylene diamine (OPD) (from Sigma) in 20µl hydrogen peroxide, 0.1M citric acid and 0.2M Na2HPO4 buffer and allowed to react for 15 minutes. The enzyme reaction was terminated with 30µl per wells 2M H2SO4 for 5 minutes. Optical densities (OD) of wells of microtitre plates were measured in a Dynatech ELISA reader (model MR4000) at 492nm-test filter and 630nm-reference filter.

#### Inhibition (competitive) ELISA

SDS extract was used at 1:1000 and serum at 1:50, for Cam 8, and 1:25 dilutions for Cam 22 and Cam 28. After the serum step, mAb was added at 1:25 dilutions. All subsequent steps were the same for isotype assay.

#### Assay control

Optimum concentration of antigens, serum, monoclonal antibodies, and conjugates were determined in a series of pre-titration experiments. The final assays were performed in duplicate. Percentage inhibition was based on the difference between individuals mean OD-value and mean±2SD of the 16 internal control blank wells.

#### Statistical analyses

The means and standard deviation (mean±SD) for absolute and percentage changes over pretreatment values for group or subgroup were tabulated. The differences between pre-treatment and post-treatment values were subjected to t-test of paired and unpaired data.

#### RESULTS

# Changes in ELISA antibodies after treatment

Percentage increases in antibodies were calculated as the difference between baseline (pre-treatment) and follow-up (post-treatment) optical density (OD)values over the baseline value. Among the antibody classes, the only remarkable increase was in IgG reactivity. Similarly, the IgG subclasses had increases as shown on Table 1. A change of ten percent and above (≥10%) were recorded in 16 individuals (50%), nine (28.2%) and four (12.5%) of the samples for IgG, IgM and IgA responses to SDS extract, respectively. Among the three IgG isotypes, levels were enhanced in 13 (40.7%) patients for both IgG1 and IgG4, and 8 (25%) for IgG3. The

mean±SD of IgG, IgG1 and IgG4 increased from  $0.60\pm0.15$ ,  $0.42\pm0.11$  and  $0.46\pm0.09$  pre-treatment OD-values to  $0.68\pm0.14$ ,  $0.45\pm0.10$  and  $0.50\pm0.07$  posttreatment OD-values. The increases represented a percentage change of  $15.1\pm24.9\%$ ,  $11.1\pm22.5\%$ , and  $13.0\pm25.4\%$ . On the contrary, decreases of  $\geq10\%$  were recorded in 16 (50%) of the samples for IgA and 8 (25%) for IgM and 7 (22.0%) for IgG3.

#### Analysis by sex and age

A significant increases in IgG, IgG1, and IgG4 antibodies in female subgroup (n=19) were higher than the male subgroup (n=13) as shown on Table 1 (P<0.05). Only IgG antibody showed an age dependent increase of 10.2±15.3%  $(0.62\pm0.16 \text{ vs } 0.66\pm0.12)$  for those  $\leq 40$ year (n=16). The 19.6±31.1% (0.58±0.14 vs 0.69 $\pm$ 0.16) for individuals  $\geq$ 41 year old (n=16) was significant. However, the differences between the two subgroups ( $\leq$ 40 year and  $\geq$ 41 year old) were not statistically significant (P>0.05).

Analysis of antibody changes by infection status

There were no remarkable differences between pre- and post-treatment IgG and IgG1 levels. Only IgG4 antibody was significantly higher in the subgroup with (n=16) than without (n=16) palpable nodule (Table 2). Presence of palpable nodule had affected IgM antibody increase in those with 10.4±19% as against the 1.0±29.7% for those without nodule. Similarly, only IgG4 antibody level was increased among persons having high skin microfilariae  $(\geq 16 \text{mf/snip},$ n=14)) compared to the subgroup with low skin mf ( $\leq 16$  mf/snip, n=18). The percentage difference of 21.9±35.8% vs. 5.6±6.1%, respectively was statistically significant by t-test of unpaired data (P<0.05).

# Evaluation of binding of mAbs to worm extract

The increased capacity of serum to inhibit binding of three monoclonal antibodies designated Cam 8, Cam 22, and Cam 28 to SDS extract was evaluated. The mean±SD of OD-values before and after treatment were 0.21±0.04 vs. 0.19±0.06) for Cam 8, with 0.39±0.12 vs. 0.34±0.14 for Cam 22 and no change in serum inhibition of Cam 28 ( $0.31\pm0.10$ ). Post-treatment changes in serum inhibition of  $\geq 10\%$  occurred in 13 (40.7%) for Cam 8, with Cam 22 having 15 (48%

# Effect of onchocercal skin disease on antibody levels

Presence of onchocercal skin disease (OSD) in individuals influenced the levels of antibodies. Table 3 shows that those presenting with OSD (n=14) had increased IgG1 and IgG4 than those without OSD (n=18). The reverse was the case for IgG antibody with a mean+SD percentage change of 20.5±29.5% and 7.7±13.9% for those without OSD compared to those having OSD. A change in IgG mean±SD of ODvalues before and after treatment from  $0.59\pm0.15$  to  $0.68\pm0.13$ , was statistically significant by t-test of paired data (P<0.05).

#### Serum inhibition

Cam 28 had 8 (25%) of the samples (n=32). An age dependent increase in inhibition of Cam 22 was recorded, but

not statistically significant (P>0.05). In addition, the difference was influenced by presence of nodule and number of skin microfilariae. Increase in Cam 22 inhibition was more in subgroup without OSD compared to Cam 8 that had higher increase in the subgroup having OSD.

Mean±SD	IgG	IgG1	
			IgG4
Pre-dose (n=32) Post-dose Percentage changes (%)	0.55±0.15 0.65±0.16 20.5±28.4*	0.39±0.11 0.44±0.12 19.0±25.4*	0.43±0.10 0.49±0.08 18.2±30.6*
Pre-dose, male (n=13)	0.67±0.13	0.47±0.10	0.50±0.05
Post-dose	0.71±0.09	0.46±0.09	0.52±0.04
% changes	9.4±15.11	No change**	4.3±6.8**
Pre-dose, female (n=19)	0.562±0.15	0.39±0.11	0.43±0.10
Post-dose	0.65±0.16	0.44±0.12	0.49±0.08
% changes	18.63±29.16*	18.6±25.5**	18.5±31.0**

Table 1: Changes in antibodies two months after ivermectin treatment analyzed by sex.

Note: \*Observed intra difference within a subgroup (pre-dose vs post-dose) and \*\*inter differences between subgroups (male and female) were statistically significant (P<0.05) by t-tests of paired and unpaired data, respectively. SD= standard deviation.

 Table 2:
 Changes in antibodies two months after ivermectin treatment depending on palpable nodule.

Mean±SD	IgG	IgG1	
	-	-	IgG4
Pre-dose nodule -ve (n=16)	0.59±0.17	0.41±0.13	0.46±0.09
Post-dose	0.65±0.16	0.42±0.11	0.49±0.09
Percentage change	13.8±32.3	10.3±28	7.3±12.4
Pre-dose nodule +ve (n=16)	0.62±0.12	0.43±0.10	0.46±0.09
Post-dose	0.70±0.11	0.48±0.10	0.52±0.04
Percentage changes	16.0±14.1*	11.5±15.2	18.1±32.8*

Note: \* Intra differences in antibody levels were statistically significant (P<0.05) by t-test of paired data. The -ve = negative and +ve = positive subgroups

Mean±SD	lgG		IgG4
		IgG1	
Without OSD (n=18) Before treatment	0.59 ± 0.15	$0.41 \pm 0.11$	$0.47 \pm 0.07$
After treatment	0.67 ± 0.12	$0.43 \pm 0.11$	0.50 ± 0.06
% changes	20.5 ± 29.6*	7.0 ± 19.7	8.0±11.4
With OSD (n=14) Before treatment	0.63±0.14	0.43 ± 0.12	$0.45\pm0.10$
After treatment	0.68 ± 0.16	$0.47\pm0.10$	$0.50\pm0.08$
% changes	7.7 ± 13.9	16.0 ± 24.8*	18.8 ± 35.4*

# Table 3: Changes in antibodies after treatment analyzed by presence of onchocercal skin diseases.

Note: OSD= Onchocercal skin diseases. \*The intra differences were statistically significant (P<0.05) by t-test of paired data.

#### Discussion

Antibodies to *O. vulvulus* adult worm extract were quantified in ELISA in same patients' (n=32) paired sera taken before and two months after initial ivermectin dosing. Appreciable increase in antibody levels that were observed in some and not in others only confirmed the variability within an individual, and individual differences in immune response to onchocerciasis infection

(6). The poor performance of IgA followed by IgM and IgG3 antibodies clearly point to non-stimulation or specific tolerance by many infected individuals. Other possibilities include the fact that IgA and IgM have relatively shorter half-life and they are produced in low concentration. While IgM is mainly a primary response compared to the secondary or anamnestic response induced by ivermectin treatment. In addition, the rates at which the three antibodies were catabolised must have by far outweighed the rates of their syntheses. The combination of these factors is strongly believed to underlie the decreases recorded for the three antibodies. Yet, no remarkable trend was exhibited by the observed decline. The enhanced antibody titers in females than the males following treatment

could not be explained. Whether this difference connoted gender in immunological reaction to the drug calls for further investigation. For long, gender related differences to infection, immune responses, and clinical manifestations have all been suspected. Our data clearly showed that parasite materials librated after ivermectin treatment evokes variable secondary or anamnestic responses resulting from antigenic stimulation.

A large number of microfilariae from superficial layers of skin were reported to be sequestered into deeper connective tissues, fats and lymph node following treatment (10). This might be one reason why vigorous responses do not ivermectin accompany treatment. Previous research works undertaken along this line have shown changes in qualitative immunoblot analyses of IgG and IgE antibody to previously recognized and unrecognized antigens, which occurred in 50% and one third of the patients, respectively. Others (3) had observed the same frequency of antibody change two weeks after diethylcarbamazine (DEC) and

ivermectin treatment, respectively. From our study, a similar quantitative trend two months post-treatment was established which 50% in of onchocerciasis patients presented with immune antigen-specific response. Whether the observed increases in antibodies provoke concomitant immunity in those individuals remains a matter of conjecture.

On the contrary, the other specter of exhibited individuals immune unresponsiveness or tolerance. Reports showed that acquisition of O. volvulus could not occur constantly over time, which strongly emphasize the immunosuppressive importance of processes in man. Suppression of parasite-specific immunity leads to parasite establishment rates, which increase along with the parasite burden (11). Therefore, we can only speculate that there was B-cell tolerance to antigens released after treatment, since not all individuals responded with Normally, if increased antibodies. antigen is present in high enough concentration for a long period it is

possible for immune tolerance be induced following a state of specific immunity (12). In chronic infection, clonal exhaustion may diminish the capacity of immune system to respond following treatment (3). Circulating antigens that form complexes with antibodies in onchocerciasis (1 & 13) were capable of inducing some form of tolerance. All these notwithstanding, what remains unclear are whether the assumed state of tolerance involved Tlymphocytes in clonal elimination or blocking. Already, Steel et al., (5) have shown that T cell proliferative response was enhanced 6 months after ivermectin reverted treatment but back to pretreatment values in 1year. It is very likely that the adult worm extract used contains mostly low molecular weight (LMW) antigens (9). The antigens could be either proteins or polysaccharideprotein complexes that can provoke Tdependent antibody responses.

The remarkable increases in IgG4 antibody associated with infection intensity based on skin microfilaria density and palpable nodule were statistically significant (P<0.05). The increase was apparently in agreement with earlier reports correlating the levels of this isotype with parasite burden (14-15). It seems IgG4 is a likely candidate of choice for antibody assays for drug screening. Again, it plays the role of blocking hypersensitivity reaction induced by IgE and preventing microfilaria clearance as well (7, 16, 17 & 18). Earlier work by Whitworth et al., (19) showed there was no reduction in itching 2 months after initial ivermectin treatment. Higher increases in IgG1 and IgG4 antibody level of patients having skin diseases support the fact that hypersensitivity induced by the former is unaffected by immune blockade of the later.

Among the three mAbs, only Cam 22 showed an appreciable overall increase in percentage inhibition. The observed increases in older age ( $\geq$ 41years) and palpable nodule positive subgroups compared with those of younger age ( $\leq$ 40years) and palpable nodule negative subgroups respectively, were not statistically significant (P>0.05). Important inferences from this study are (i) the gender sensitivity to antibody changes, (ii) association of IgG1 and IgG4 with skin disease, and (iii) the potential of IgG4 indirect and Cam 22 competitive ELISA for drug screening. In addition, the studies showed there selective unresponsiveness to was antigenic epitopes defined by the mAbs. In future, this type of study will become difficult to carry out, since most endemic areas would have been covered by the on-going community directed treatment of onchocerciasis with ivermectin.

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#### **ORIGINAL ARTICLE**

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# PHARMACOECONOMIC EVALUATION OF DOXYCYCLINE AND TETRACYCLINE IN THE TREATMENT OF CHLAMYDIAL IMPLICATED NON-GONOCOCCAL URETHRITIS IN A TERTIARY HEALTHCARE INSTITUTION IN NIGERIA.

\* Giwa A<sup>1</sup>, Osagbemi GK<sup>2</sup>, Olayinka BO<sup>3</sup>, Giwa HBF<sup>4</sup>. <sup>1</sup>Department of Clinical Pharmacy and Pharmacy Administration, faculty of Pharmacy, University of Maiduguri, Nigeria. <sup>2</sup> Department of Epidemiology and Community Health, College of Health Sciences, University of Ilorin, Nigeria; <sup>3</sup> Department Pharmaceutics and Pharmaceutical Microbiology, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria, Nigeria and <sup>4</sup> Department of Pharmacy, University of Ilorin Teaching Hospital, Nigeria

\* Correspondence Email: abdulganiyugiwa@yahoo.com

#### ABSTRACT

With depressing nature of economy in many countries such as Nigeria where per capita income is low, there is need for utmost consideration for cost containment measures.

Objective: The objective of this study is to conduct pharmacoeconomic evaluation of two antichlamydial indicated non-gonococcal urethritis therapeutic options and to make recommendations for inclusion of economic evaluation of drug therapies in health policy formulations and decision making.

Methods: Cost effectiveness analysis was carried out retrospectively for prescribed/dispensed antibacterials to out-patients with chlamydial implicated non-gonococcal urethritis among other infectious diseases, by examining out-patient case notes between 2005 and 2007 in Ahmadu Bello University Teaching Hospital, Zaria Nigeria.

Results : The result shows that doxycycline costs N1.33/unit of effectiveness while tetracycline costs N2.77/unit of effectiveness in the treatment of chlamydial implicated non-gonococcal urethritis. Doxycycline is therefore more cost effective than tetracycline capsules. Subjecting the costs and effectiveness to sensitivity analysis did not change this conclusion. There is statistically significant difference in the effectiveness (outcome) of doxycycline (78.8%) and tetracycline (58.7%) (x<sup>2</sup> =9.4; p<0.05) There is therefore association between effectiveness and therapeutic option chosen with doxycycline being a more cost-effective option. The result is significant because doxycycline is not currently included in the Essential Drug list of Nigeria<sup>1</sup>. However, the result is in agreement with Zimbabwean Essential Drug list which recommended that tetracycline be replaced by doxycycline in all indications and should be used only when doxycycline is not available<sup>2</sup>. Also doxycycline is a drug of choice for other disease like gonorrhorea and syphilis in non-pregnant women<sup>2</sup>. It was concluded that Doxycycline 100mg bd x  $\frac{1}{52}$  is more cost effective than Tetracycline 500mg qid x  $\frac{1}{52}$  in the treatment of chlamydial implicated non-gonococcal urethritis. Adoption of economic evaluation of drug therapies in Nigeria Health policy formulation and decisions is likely to enhance overall Health System cost effectiveness.

KEYWORDS: Pharmacoeconomics, Cost effectiveness analysis, Doxycycline, Tetracycline, Nongonocccal Urethritis.

#### **INTRODUCTION:**

The advancement in medical technology (both diagnostic and therapeutic options) have complicated the financial burden of Health Systems. Although they offer the potential to improve quality of care, these advances have significantly increased Health Systems operating costs. In spite of all aforementioned inherent and obvious predicaments, public expectation from healthcare providers and government is increasingly becoming higher on a daily basis (3, 4).

Therefore, there is need for a useful scientific intervention that can facilitate rational decision-making. motivating healthcare practitioners to consider more compressive model for medical decision-making. All these trends have led to the evolution of Pharmacoeconomic, a relatively new discipline in Pharmacy (5, 6).

Cost-effectiveness analysis, a form of Pharmacoeconomics tool appears more effective if applied properly in The therapeutic decision-making. various outcomes of therapy namely; economic, clinical and humanistic (psychosocial) are considered (7). A comparative cost and outcome evaluation carried out for was Doxycycline and Tetracycline in the treatment of chlamydial implicated nongonococcal urethritis in Ahmadu Bello University Teaching Hospital, Zaria, Nigeria.

#### MATERIAL AND METHODS

Time and motion studies in conjunction with standard cost-accounting techniques were used in this retrospective study.

#### Patients

The analysis addressed adult outpatients in Out-Patient Department Ahmadu Bello University Teaching Hospital, Zaria, Nigeria with chlamydial implicated non-gonococcal urethritis among other infectious disease confirmed by necessary diagnostic tools (Table 3).

Consequently, interest in research to assess the outcomes of healthcare has been on the increase. Medical, ethical and societal concerns about costs, access and quality of care are also **Data Collection** 

One thousand and eighteen (1018) outpatient case notes for selected diseases were consecutively examined using diagnostic coding cards. These were essentially diseases that had antibacterial agents as the mainstay of therapy. One hundred and forty eight (148) of the patients suffered from chlamydial implicated non-gonococcal urethritis.

In all, 1527 dispensed prescriptions were sampled systematically and examined. Relevant information were extracted and recorded. These included prescribed/dispensed drugs between year 2005 and 2007. Others were dosage, duration of therapy, patient demographic data, diagnosis, concurrent illness, diagnosis tests ( if any), physician's remark on each visits and cost of drugs.

#### **Computation of Data**

The cost per Defined Daily Dosage (DDD) of each antibacterial was DDD calculated. units are by World Health recommended Organization (WHO) for analysis of drug use. DDD represents the usual daily dosage of antibacterial per day (8).

#### **Cost-effectiveness Analysis**

Analysis of costs in monetary units and effectiveness in natural units (eradication of bacteria and clinical cure) were carried out.

# Conduction of cost-effectiveness analysis (9, 10)

*Definition of Pharmacoeconomic problem:* Should option I or II be recommended as therapy of choice for the treatment of chlamydial implicated non-gonococcal urethritis? (Table 3)

*Definition of the goals and objective of problem situation:* The objective was to determine which of the treatment options provide greater value for money for using effectiveness rating (Table 4), decision analysis(Table 5) cost of therapy (Table 6) and cost effectiveness analysis table (Table 7).

*Perspective:* Economic perspective of the Health institution was chosen since the drugs were prescribed there. However, patient perspective was considered where necessary.

*Enumeration of the different ways to achieve the objective:* (Table 4) Consideration of available/preferred treatment options was made between doxycycline and tetracycline.

Determination of cost of therapy

Only direct medical costs were included in the analysis. These included overhead and operating costs such as acquisition costs of drugs, staff time (costs associated with preparation and dispensing of the product) where it differs from the two options considered. Others include equipment, disposables and transport costs to patients where applicable. The cost per defined daily dosage (C/DDD) of each drug was used (Table 6).

Time and motion studies were carried out for Pharmacists that differ between each option. There was no statistically significant difference between the frequencies of Physician visits among the two treatment options considered, being outpatients.

The time and motion studies involved observing the actual work of each personnel including dispensing of capsule. Each activity was timed using a stopwatch and the average time for 10 random observations for the completion of the task was determined.

The mean salary for the healthcare personnel was obtained from the Accounts section of the hospital.

Mean salary/sec =

annual salary

Hours/week x Number of weeks/annum x 360

The individual costs were converted into cost per dosage regimen.

**Discounting**No adjustment for inflation or discounting was made for the analysis. Costs were fairly stable and both options were used within each year under review. However, slight variation over the period under review in some cases led to the use of mean cost of each option.

Consequences (Outcomes of each treatment option).

The literature was reviewed for positive and negative outcomes of each treatment options (Table 4) (11-14)

#### Sensitivity Analysis

Sensitivity analysis was performed to test whether the decision changes when specific variables altered within reasonable range in favour of less cost effective option. This was carried out for the cost of treatment options and effectiveness (Table 8)

#### Data Analysis

Statistical analysis was carried out on the results obtained. The effectiveness rating (percentage, proportion) was compared by the use of Chi-square analysis.

#### RESULTS

#### Table 1: COST EFFECTIVENESS ANALYSIS (CEA)

	Cost of therapy	Effectiveness (E)	CEA (%)
Doxycycline Option I	N104.3	78.8	N1.33/Unit of effectiveness
100mg b.d x <sup>1</sup> / <sub>52</sub>			
Tetracycline (Option II)	N62.5	58.7	N2.77/Unit effectiveness
500mg q.i.d x <sup>1</sup> / <sub>57</sub>			

Using Doxycyline capsule (option I) in the treatment of chlamydial implicated non-gonococcal urethritis at a full course of 100mg bd x 1/52 cost N104.3 with effectiveness measure of 78.8 and cost effectiveness of N1.33/unit of effectiveness. While tetracycline capsules at a full course of 500mg qid x  $\frac{1}{52}$  (option II) when used for treatment of same condition cost N162.5 with effectiveness measure of 58.7 and cost effectiveness of N2.77/unit of effectiveness.

Doxycyline capsules 100mg qid 1/52 is therefore cheaper per unit of effectiveness than tetracycline capsules 500mg qid x 1/52 in the chlamydial implicated non-gonococcal urethritis and therefore more cost effective.

There is statistically significant difference in the effectiveness (outcome) of Doxycyline and tetracycline (58.7%) ( $X^2 = 9.4$ ; p< 0.05). There is therefore association between effectiveness and therapeutic option chosen.

Table 2: SI	ENSITIVITY	ANALYSIS
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S/NO	ALTERATION IN VARIABLE	COST EFFECTIVENESS
1	Increasing the cost of doxycycline by 50%	N1.99/unit of effectiveness
2	Decreasing the cost of doxycycline by 40%	N1.66/unit of effectiveness
3	Increasing the effectiveness of tetracycline to 78.8 (Doxycyline value)	N2.006/unit of effectiveness

Sensitivity analysis (what if analysis) indicates the decision still remains

valid, confirming doxycycline to be more cost effective, despite alterations

Diseases condition	Diagnostic tools	Treatment option	
Non-gonococcal urethritis where	Objective evidence of urethra	Doxycycline capsules	Tetracycline capsules
chlamydial is implicated	discharge (Expressed by milking or	100mg bid x <sup>1</sup> / <sub>52</sub>	500mg qid x <sup>1</sup> / <sub>52</sub>
	$\geq$ 5PMIN/100 x field in the urethra		
	secretion, with the exclusion of N.		
	gonorrhea by Gram's stain and/or		

## Table 3: Treatment Options For Cost- Effectiveness Analysis

culture

# TABLE 4: EFFECTIVENESS RATING

CRITERIA	CAP DOXYCYCLINE (OPTION L)	VALUE	CAP TETRACYCLINE	VALUE
Spectrum of activity Assumption	Broad-spectrum antibiotic. Gram negative Gram-positive organisms and chlamydial are sensitive, as well as 70-90% of anaerobes. More active for resistant organisms 100% sensitivity of chlamydial infection assumed for both options in their respective dosages	100%	Broad-spectrum antibiotic. Gram negative Gram-positive organisms and chlamydial are sensitive, about 10% resistance for Gram-positive and 50% for some Gram-negative organisms reported, less active.	100%
2a Pharmacokinetics 2b Frequency of	Oral absorption93%Pre-system metabolismNilBioavailability93%Plasma t½ (range)18-22hrsPlasma protein binding82-93%	93%	Oral absorptionirregular&incompletePre-system metabolismNilBioavailability60%Plasma t <sup>1</sup> /2 (range)8-5hrsPlasma protein binding36-50%	80%
administration	U.a. or b.a	75%	Qid	25%
3. Interaction	It can be taken with food or milk if gastric irritation is a problem although chelates multivalent cations the least for the tetracycline	50%	It cannot be taken with food or milk because of irregular and incomplete oral absorption. It is a chelationg agent for multivalent cations	1%
4. Adverse Drug Reaction (ADR)	Nausea Vertigo Rash Phototoxicity Safe in renal insufficiency Each negative effect assumed to be 10% Tolerability = 100-40	60%	Nausea Vertigo Rash Phototoxicity Less safe in renal insufficiency Tolerability = 100-50	50%

Notice o.d =100%, bid = 50%, qid = 25%

CRITERIA	CAP. DOX	CAP. DOXYCYCLINE (OPTION I)		Cap. Tetracycline (Option II)		
	Value (%)	Assigned Weight	Criterion rating	Value (%)	Assigned Weight	Criterion rating
1. Spectrum of activity	100	0.4	40	100	0.4	40
2a Pharmacokinetics	93	0.1	9.3	60	0.1	6.0
2b. Frequency of administration.	75	0.1	7.5	25	0.1	2.5
3. Interaction						
4. Tolerability (100-DR%)	50	0.2	10.0	1	0.2	0.2
	60	0.2	12.0	50	0.2	10
SUM OF CRITERIA RATINGS		1.00	78.8		1.00	58.7
(EFFECTIVENESS MEASURE)						

## Table 5: Table Of Decision Analysis.

#### **TABLE 6: Cost Of Therapy**

Only direct medical costs, which included; drug acquisition cost and costs associated with dispensing were considered.

OPTION I	OPTION II
DOXYCYCLINE CAPSULES	TETRACYCLINE CAPSULES
Acquisition Cost = C/DDD x DOT in days = 100mg	Acquisition Cost = 500mg qid x <sup>1</sup> / <sub>52</sub>
bid x $\frac{1}{52}$ - 14 x 7 = 98.00	$= 20 \times 7 = N140.00$
Cost of dispensing by Pharmacist = Mean salary/sec x time taken for dispensing ins seconds = 0.2680 x 24 = N6.43 Total cost = N104.43	Cost of dispensing by Pharmacist = 0.2680 x 84 = N22.51 Total Cost = N162.51

Physician office visit and patient traveling cost is assumed to be the same for both options.

#### Table 7: Cost Effectiveness Analysis (CEA)

OPTION I DOXYCYCLINE CAPSULES	OPTION II TETRACYCLINE CAPSULES
Cost = 104:43, Effectiveness = 78.8	Cost = 162:51, Effectiveness = 58.7
$CEA = \frac{104:43}{78.8} = N1.33/unit of effectiveness$	$CEA = \frac{162:51}{78.8} = N2.77/unit of effectiveness$

#### **Table 8 : Sensitivity Analysis**

i. Increasing cost of doxycycline by 50% (156.65)
CEA <u>156.65</u> = N1.99 unit of effectiveness
70.0
ii. Decreasing cost of retracycline by 40 /0 (97.51)
CEA <u>97.51</u> = N166 unit of effectiveness 58.7
iii. Increasing the effectiveness of tetracycline to 78.8 (Doxycycline value)
CEA <u>162.51</u> = N2.06 unit of effectiveness 78.8

Sensitivity analysis ("what if') indicates that the decision still remain valid, confirming doxycycline to be more cost effective.

#### DISCUSSION

The result of this study is significant because the Essential Drug List of Nigeria does not include doxycycline<sup>1</sup> in spite of its cost-effectiveness over tetracycline as shown in this study.

With NAFDAC's recent commitment to registration of good quality drugs, economic evaluations in form of pharmacoeconomic trials reports should be required along with clinical trials report for the inclusion of a drug on the Essential Drugs List. With these, decisions for drug registration will be based on the principle that "if a drug is not better than a comparable product, it should not cost more. If it is superior to existing therapies but more expensive (a common situation), and funds are available, any extra expenditure should represent "value for money" (15).

The result of this study is in agreement with Zimbabwean Essential Drugs List which recommended that tetracycline be replaced by doxycycline in all indications, and should only be used when Doxycyline is not available (2). Also Doxycyline is a drug of choice for other disease like gonorrhea and syphilis in non-pregnant women<sup>2</sup>. This result is also similar to the outcome of a comparative study for doxycycline and co-trimoxazole in the non-gonococcal urethritis where conclusion was drawn that Doxycyline is superior to cotrimoxazole and may become the drug of choice for uncomplicated nongonococcal urethritis (16). The findings of this study is in agreement with the report of the National Network for

STD/HIV Prevention and Training Center that doxycycline at a course of 100mg bid x 1/52 for treatment of Chlamydial infection has an efficacy of 95-100%, lower cost and better tolerability than Erythomycin 2mg daily x 1/52 with 85-95% efficacy, higher cost and only fair tolerability (17)

The statistically significant difference in the effectiveness of Doxycycline (78.8%) and tetracycline (58.7%) ( $X^2$ = 9.4; p<0.05) appear to be due to difference in their economic clinical and humanistic outcomes (7).

Doxycyline achieves bioavailability of 93% after oral absorption while tetracycline due to irregular and incomplete oral absorption has 60% bioavailability (11). This and once daily or twice daily dose of doxycycline as compared with four times daily dose of tetracycline, significantly favours the effectiveness rating of doxycycline over tetracycline. The fact that tetracycline cannot be taken with food or milk as compared with doxycycline which can be taken with food or milk if gastric irritation is a problem (14), also favours the effectiveness of doxycycline over tetracycline. Doxycycline is also the least affected by the multivalent reports of resistance to tetracycline. In terms of tolerability, doxycycline is reported to be safer in renal insufficiency than tetracycline(11, 12, 13). This also of enhances effectiveness rating doxycycline in cost-effectiveness analysis.

# CONCLUSION AND RECOMMENDATIONS

The results of this work has shown that doxycycline capsules at a course of 100mg bid x  $^{1}/_{52}$  is more cost effective than tetracycline capsules at 500mg qid x  $^{1}/_{52}$  for the treatment of chlamydial implicated non-gonococcal urethritis at p<0.05. This is enough justification for ensuring that doxycycline is included in the revised Nigerian Essential Drug List. Adoption of economic evaluation of drug therapies in National health policy formulation and decision is therefore likely to guarantee the overall Health System cost-effectiveness.

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## **ORIGINAL ARTICLE**

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# COMPARATIVE COST-EFFECTIVENESS ANALYSIS OF STREPTOMYCIN AND ETHAMBUTOL IN THE TREATMENT OF TUBERCULOSIS IN A UNIVERSITY TEACHING HOSPITAL IN NIGERIA.

\* Giwa A<sup>1</sup>, Osagbemi GK<sup>2</sup>, Olayinka BO<sup>3</sup>, Giwa HBF<sup>4</sup>. <sup>1</sup> Department of Clinical Pharmacy and Pharmacy Administration, Faculty of Pharmacy, University of Maiduguri, Nigeria; <sup>2</sup> Department of Community Health and Epidemiology, College of Health Sciences, University of Ilorin, Nigeria;

<sup>3</sup> Department Pharmaceutics and Pharmaceutical Microbiology, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria, Nigeria, and <sup>4</sup> Department of Pharmacy, University of Ilorin Teaching Hospital, Nigeria

\* Correspondence: Email: abdulganiyugiwa@yahoo.com

#### ABSTRACT

Healthcare organizations, governments and individuals have been forced by prevailing circumstances of economic crisis to be increasingly oriented towards cost containment due to escalating nature of health expenditure.

Objective:

The objective of this study is to determine the comparative cost effectiveness of various antituberculous therapeutic options and to make recommendation for the adoption of costeffectiveness evaluations in National Health Policy formulation and decision-making. *Method* 

Retrospective cost effectiveness analysis was carried out for prescribed/dispended antibiotic to outpatients with tuberculosis among other infectious diseases in outpatients case notes between 2005 and 2007 in Ahmadu Bello University Teaching Hospital, Zaria Nigeria.

Results

The result shows that ethambutol tablet cost N8.40/unit of effectiveness while streptomycin injection cost N81.50/unit of effectiveness in the treatment of tuberculosis. Ethambutol tablet therefore appears to be more cost effective than streptomycin injection. Subjecting the cost and effectiveness to sensitivity analysis did not change this conclusion. Statistical analysis shows that there is a statistically significant difference in the effectiveness (outcome) of ethambutol (95%) and streptomycin injection (76.73%) ( $X^2 = 13.75$ ; p<0.5). Therefore there is association between effectiveness and therapeutic option chosen with ethambutol tablet being a more cost effective option. The result of this study is significant because ethambutol is usually traded off for less cost-effective streptomycin in many cases even when there is no contraindication to the use of ethambutol.

CONCLUSION

Ethambutol tablet is more cost effective than streptomycin injection at their usual therapeutic doses in combination with isoniazed, rifampicin and pyrazinamide in the treatment of tuberculos<sup>1</sup> at the intensive phase.

KEYWORD: Pharmacoeconomics, cost effectiveness analysis, ethambutol, streptomycin, tuberculoses.

#### **INTRODUCTION:**

Orientation towards cost containment due to escalating nature of health expenditure is continuously increasing. Only few data also exist regarding the actual cost and benefits attributed to specific drug therapy in spite of widespread use of pharmaceuticals. This is probably due to lack of welldefined methodologies to evaluate medical intervention. Health sector capital income is low, whereas this increase in expenditure does not necessarily translate into increase per head or access.(1)

The health system is clearly in a state of rapid evolution. Traditional approaches to healthcare decisions will no longer suffice, as they are not effective in curtailing cost objectively, therefore new tools need to be employed.

Cost-effectiveness analysis, a form of pharmacoeconomic tool appears more effective if applied properly in therapeutic decision making. The various outcome of therapy namely, economic. clinical and humanistic (psychosocial) outcomes are considered (1). A comparative cost-effectiveness carried analysis was out for streptomycin and ethambutol in the treatment of tuberculosis in Ahamadu Bello University Teaching Hospital, Zaria, Nigeria.

## MATERIALS AND METHODS

A retrospective study involving time and motion studies in conjunction with standard cost accounting techniques was carried out.

Patients

allocation is increasing partly due to population growth and partly due to new health development. This trend is not only observed in developed economy but also in developing ones like Nigeria where per The study addressed adult outpatients the Outpatients Department of in Ahmadu Bello University Teaching Hospital, Zaria with tuberculosis infectious diseases among other diagnostic confirmed by necessary tools. (Table 3).

#### Data Collection

A total of 1018 outpatient case notes for tuberculosis were consecutively examined using diagnostic cards. These are essentially diseases that have antibacterial agents as the

mainstay of therapy. One hundred and ten (110) of the patients suffered from tuberculosis.

A total of 1527 dispended prescription were sample systemically and Relevant information on examined. prescribed/dispended drugs between the year 2005 and 2007 were extracted and recorded. These included patient demographic data, diagnosis, concurrent illness, diagnostic test (if any), drug prescribed, dosage, duration of therapy, physician's remarks on each visit and cost of drugs as well as treatment outcome.

#### Computation of Data

The cost per Defined Daily Dosage (DDD) of each antibacterial was calculated. DDD units are recommended by World Health Organization (WHO) for analysis of drugs use. DDD represents the usual dosage of an antibacterial per day (e.g Ampiclox 2g per day in 4 divided doses) (2).

#### **Cost-effectiveness Analysis**

Analysis of cost (in monetary units), and effectiveness in natural units (eradication of bacteria and clinical cure):

# Conduction of Cost-Effusiveness Analysis (3, 4)

# Definition of Pharmacoeconomic problem

Should Option I be recommended or Option II (Table 3) as therapy of choice for the treatment of tuberculosis?

# Definition of the goal and objectives of problem situation

The objective is to determine which of the treatment options provide greater value for money using effectiveness rating (table 4), decision analysis (Table 3), cost of therapy (Table 6) and coasteffectiveness analysis (table 7)

#### Perspective

Economic perspective of the health institution was chosen since the drugs were prescribed there. However, patient perspective was considered where necessary

> a. Enumeration of the different ways to achieve the objective (Table 4)

> > Consideration of valuable/ preferred treatment options.

b. Determination of Costs of therapy

Only direct medical costs were included in the analysis. These include overhead and operating costs such as acquisition costs of the drugs. Staff time (costs associated with preparation, dispensing, administration of product) where it differs from the two options considered. Others include equipment, disposa and transport costs to patient. The cost per defined daily dosage (c/DDD) of each drug was used (Table 6)

Time and motion studies was carried out for Pharmacists and Nurses that differed between each option. There was no significant statistically difference between the frequency of physician visits among the two treatment options considered being outpatients. The time and motion studies involved observing the actual work of each personnel. This included the preparation and administration of injection and dispensing of tablets. Each activity was timed using a stopwatch and the average time for 10 random observations for the completion of each of the tasks was determined. The mean salary for the healthcare personnel was obtained from the accounts section of the hospital and calculated as follows:

Mean salary/sec =

Annual Salary

Hrs./wk x No. of wrks/annum x 360

The individual costs were converted into cost per dosage regimen.

### Discounting

No adjustment for inflation or discounting was made for the analysis. Costs were fairly stable and both options were used within each year under review. However, slight variation over the period of time required in some cases led to the use of mean cost of each option.

*Consequences* (Outcomes) of each treatment option.

The literature was reviewed for positive and negative outcomes of each treatment options (Table 4) (4-9)

#### Sensitivity Analysis

Sensitivity analysis was performed to test whether the decision changes when specific variables altered within reasonable range in favour of less cost effective option. This was carried out for the cost of treatment options and effectiveness(Table 8)

#### Data Analysis

Statistical analysis was carried out on the results obtained. The effectiveness rating (percentage, proportion) was compared by the use of Chi-square analysis.

#### RESULTS

Table 1: COST	EFFECTIVENESS	5 ANALYSIS	(CEA):-
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	Cost of therapy	Effectiveness (E)	CEA (¢/ <sub>E</sub> )
Ethambutol Tablet 400mg b.d. x <sup>3</sup> / <sub>12</sub> (Option I)	N798.183	95	N8.40/Unit of effectiveness
Streptomycin Inj. 1gm o.d. x <sup>3</sup> / <sub>12</sub> (Option II)	N6,253.80	76.73	N81.50/Unit effectiveness

Using Ethambutol Tablet (option I) in combination with Isoniazid, Rifamficin and pytazinamide at the phase I (intensive phase) of Tuberculosis chemotherapy as a course of 400mg bid x  $^{3}/_{12}$  cost N798.12 with effectiveness measure of 95 and cost effectiveness of N8.40/unit of effectiveness while streptomycin injection as an alternative option at a course of 1gm o.d x  $^{3}/_{12}$  cost N6,253.80 with effectiveness measure of 76.73 and cost effectiveness of N81.50/unit of effectiveness.

Ethambutol tablet 400mg bid x  ${}^{3}/{}_{12}$  is therefore cheaper per unit of effectiveness than streptomycin injection 1gm o.d x  ${}^{3}/{}_{12}$  when used in combination with Isoniazed, Rifampicin and pyrazinamide in the Phase I(Intensive phase) chemotherapy of tuberculosis. There is statically significant difference in the effectiveness (outcome) of ethambutol (95%) and streptomycin injection (76.73%) ( $X^2 = 13.75$ ; p<0.05). Therefore there is association between effectiveness and therapeutic option chosen.

S/NO	ALTERATION IN VARIABLE	COST EFFECTIVENESS
1	Increasing the cost of Ethambutol tablet by 300%	N33.61/Unit of effectiveness
2	Increasing the effectiveness of streptomycin to 95% (Ethambutol value)	N65.83/Unit of effectiveness
3	Decreasing the cost of streptomycin by 50%	N40.75%/Unit of effectiveness
4	Decreasing Nurse's preparation and administration time of streptomycin to 30 sec/day	N66.60%/Unit of effectiveness

#### Table 2: SENSITIVITY ANALYSIS

Sensitivity analysis (what if analysis) indicates that the decision still remain valid as ethambutol is still more cost effective than streptomycin despite alterations made in favour of less cost effective Streptomycin.

#### DISCUSSION

Antimicrobial agents constitute the largest group of drug purchased in many countries and account for the highest proportion of drug budget <sup>7,8</sup>, therefore efforts to ensure greater cost effectiveness is indispensable in view of limited resources. Studies have shown that both ethambutol and streptomycin are predominantly used to prevent emergence of resistant strain of *Mycobacterium tuberculosis*, the causative agent of tuberculosis (7, 8, 9, 10)

This justifies their inclusion in the intensive phase (Phase I) of treatment where either of them could be used based on cost and outcome of therapy (economic, clinical and humanistic) and individual patient peculiarity. The use of streptomycin injection was found to be very rampant while ethambutol table is seldom used, even when there is no contraindication to its use in the study setting in spite of being more cost effective. This result can be used as a tool to change the prescribing habit of doctors to a more rational one. This is in agreement with the objective of pharmacoeconomic study that makes a person or a group changes their behaviour and persuade them hat a new course of action is a 'better' one. 'Better' simply means in economic terms, it is more cost efficient <sup>11</sup>. The result of this study agrees with the report of the British National Formulary that streptomycin is no longer popular as Phase I anti-tuberculous drug in many developing countries (12). The statistically significant differences in the effectiveness of Ethambutol (95%) and Streptomycin injection (76.73%) (X<sup>2</sup>= 13.75; p<0.05) could probably be due to differences in their economic clinical and humanistic outcomes7.

Ethambutol tablet being an oral preparation has no risk of infection, abscess or pain at the site of injection. It therefore achieves 100% benefit of safety of administration compared with average of 33.7% for streptomycin injection's documented risk of infection (50%), risk of abscess (50%), pain at site of injection (99%) with only 1% likely to be free from pain  $^{5,6,10.}$ 

DISEASE CONDITION	DIAGNOSTIC TOOLS	TREATMENT OPTION		
		Option I	Option II	
Pulmonary Tuberculosis	Matoux test, AFB, X-ray,	Ethambutol tab 400mg bid	Streptomycin inj 1gm o.d x <sup>3</sup> / <sub>12</sub>	
	Microscopy, culture and	<sup>3</sup> / <sub>12</sub> in combination with	in combination with	
	sensitivity (m/c/s)	Isoniazid, Rifampicin and	Isoniazid, Rifampicin and	
		Pyrazinamide	Pyrazinamide	

CRITERIA	TABLET ETHAMBUTOL	VALUE	STREPTOMYCIN INJ	VALUE
10		1000/		1000/
1.Spectrum of	Bacteriosatic with some reported	100%	Bactericidal action; intracellular lack	100%
activity	bactericidal activity (intracellular)		intracellular <sup>5,6</sup> (action). It is effective in	
			preventing the emergence of resistance	
Assumption	Both of them can achieve the desired		to other anti-tuberculous drugs but add	
	therapeutic outcome is used effectively;		little if anything to the bactericidal and	
	100% sensitivity assumed.		sterilizing action of Isoniazid,	
			Rifampicin and Pyrazinamide	
2.Pharmacokinetics	Oral absorption 80%	80%	Oral absorption not applicable	
	Pre-systemic metabolism Nil		Pre-systemic metabolism (im) Nil	
	Bioavailability 80%		Bioavailability (i.m inj. 100%	
	Plasma t½ 10-15h		Plasma t½ 2.4-9.0h	
	Frequency of administration o.d		Frequency of administration o.d	100%
Safety of	Risk infection nil	100%	Risk infection 50%}	33.7%
administration	Risk of abscess nil		Risk of abscess 50%} 66.3%	
	Pain at site of injection nil		Pain at site of injection 99%	
	Tolerability 100%		Tolerability (100-66.3)%	
A Adverse Drug	Dose dependent optic neuritis (easily	95%	Ototoxic <sup>5,6</sup> progressive damage less	50%
Reaction (ADR)	reversible) at 15mg/kg<1% at		reversible Vestibular 2.5% ditory lss	
	25mg/kg<5%.		common	
	Colour blindness Allergic rashes,		Hypersensitivity; very common 75%	
	Jaundice reported		(can as well pharmacist and nurses for	
	Tolerability (100-5)%		handling)	
			Tolerability (100-50)%	

# Table 4: Effectiveness Rating.

#### Table 5: Decision Analysis.

CRITERIA	TABLET ETHAMBUTOL (OPTION I)			INJ STREPTOMYCIN (Option II)		
	Value (%)	Assigne d Weight	Criterion rating	Value (%)	Assigne d Weight	C r i
1. Spectrum of anti tubercular activity	100	0.4	40	100	0.4	4 0
2 Pharmacokinetics	80	0.2	16.0	100	0.2	2 0
3. Safety of administration	100	0.2	20.0	33.7	0.2	6
4.Tolerability (100-DR)%	95	0.2	19.0	0	0.2	1 0
Sum Of Criteria Ratings	-	1.0	95.0	-	1.00	76.7

# COST OF THERAPY

Only direct medical costs, were considered. This include drug acquisition cost, costs associated with preparation, dispensing, administration and transport cost (to patient).

	<b>TABLE 6: Duration of therapy:</b>	Three months intensive	phase (	phase I	) treatment.
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OPTION I	OPTION II
ETHAMBUTOL TABLET	STREPTOMYCIN INJECTION
Acquisition Cost = C/DDD x DOT in days = 400mg bid $x^{3/12}$ in	Acquisition Cost =1gm o.d x <sup>3</sup> / <sub>12</sub> = (C/DDD x DOT) = 25.0x84
days = 8.00 X 84 = N672.00	= 2,100=
	(N70/5gm vial, N10/needle & syr, N5/water for Inj)
Cost of dispensing by Pharmacist = =0.2680 X 135 secs = N36.18	cost of preparation and administration by Nurses = 0.1945 x 100
Transport cost by patient (three monthly trips to refill	sec/day x 84 days =1,633.80
prescription)	Transport cost by patient (N30 per trip day) for injection a
$N30/trip = N30 \times 3 = 90.00$	consideration) = N30 x 84 N2,520
Total = N798.18	
	Total N6,253.80

#### Table 7: Cost Effectiveness Analysis (CEA)

OPTION I ETHAMBUTOL TABLET	OPTION II STREPTOMYCIN INJECTION
Cost = N798.18, Effectiveness = 95	Cost = 6,253.80,
	Effectiveness = 76.73
CEA = <u>798.18</u> = N8.40/Unit of effectiveness	
95	CEA = <u>6,253.80</u> =
	78.8
	N81.50/unit of effectiveness

#### Table 8 :SENSITIVITY ANALYSIS

i. Increasing cost of Ethambutol tablet by 300% (N3192.72)
$\begin{array}{l} \text{CEA}  \underline{3192.72}_{95} = \text{N33.61 unit of effectiveness} \\ \end{array}$
i. Increasing the effectiveness of Streptomycin to 95% (Ethambutol value)
$\begin{array}{l} \text{CEA}  \underline{6253.80} \\ 95 \end{array} = \text{N65.83/unit of effectiveness} \\ \end{array}$
iii. Decreasing cost of streptomycin 50% (N3126.90)
CEA $\frac{3126.90}{76.73}$ = N40.75 unit of effectiveness
iv. Decreasing Nurses' preparation and administration time of streptomycin
injection to 30 sec/day instead of 100 sec/day. This increases cost of therapy with streptomycin
to N5110.14.
CEA <u>5110.</u> 14 N66.60/ unit of effectiveness 76.73

Sensitivity analysis ("what if") indicates that the decision still remain valid, as Ethambutol is till more cost effective.

This humanistic outcome enhances the effectiveness rating of Ethambutol tablet over Streptomycin injection. Ethambutol has also been reported to be tolerated in 95% of patie nts on it while Streptomycin injection's tolerability is estimated to be 50%.

This explains why individual patient peculiarity must be considered in choice of therapeutic option. For example, young children whose visual acuity can hardly be monitored objectively should not be given ethambutol. Also in patient with optic neuritis. The various adverse reaction of streptomycin, such as ototoxicity, nephrotoxicity, teratogenicity and hypertensivity reactions need to be considered as well.

## CONCLUSION AND

#### RECOMMENDATIONS

It is concluded that Ethambutol tablet at a course of 400mg bid x  $^{3}/_{12}$  is more

cost effective than i.m streptomycin inj. 1gm o.d. x  $^{3}/_{12}$ , each in combination with isoniazid, rifampicin and pyrazinamide at the intensive phase (Phase I) of anti-tuberculous therapy.

A very functional anti-tuberculous drug policy and evidence based treatment guidelines should be put in place if antituberculous drugs are to be used in a cost-effective manner.

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# **ORIGINAL ARTICLE**

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# THE INFLUENCE OF ENVIRONMENTAL SANITATION PRACTICES AND HYGIENE ON THE INCIDENCE OF DIARRHOEA – THE CASE OF KOFORIDUA MUNICIPALITY, GHANA

Asenso-Mensah, Emmanuel; Awoyemi, Ademola O. and Browne, E. N. L. Department of Community Health, School of Medical Sciences, College of Health Sciences, Kwame Nkrumah University of Science & Technology, Kumasi, Ghana Correspondence: Dr.A.O.Awoyemi, Department of Epidemiology & Community Health, College of Health Sciences, University of Ilorin, Ilorin, Nigeria

#### ABSTRACT

A case-control study was done using a convenience sample of 100 pairs of mothers and children (less than five years old) divided into two groups; the first group made of children with diarrhoea and their mothers (case group) and the second group made up of mothers and children who did not have diarrhoea (control).

A structured interview schedule and review of routine data were used to collect data.

Only 12.5% of children less than six months in the case group were exclusively being breastfed compared with 75% of the control group. 56% of the cases used water from unprotected wells for domestic activity while 70% of the control group used pipe- borne water. Also 24% of the case group did not cover stored water while 76% of the control group covered stored water. This demonstrated poor food and water safety.

Only 10% of cases had access to flushing water closets for human excreta disposal while the remaining 90% either used pit latrines or disposal into surrounding bushes.

It was recommended that good amenities for the disposal of refuse in the communities be provided by the government and the delivery of pipe-borne water be made more regular to the inhabitants Further, appropriate education in simple language is to be offered by health personnel especially at Ante-Natal Clinic and on radio stations and mothers are to be encouraged to put into practice what they learn about the treatment and prevention of diarrhoea.

Keywords: Sanitation, Hygiene, Practices, Diarrhoea, Koforidua

#### **INTRODUCTION**

Diarrhoea is the passing of increased amounts (more than 300g in 24hours) of loose stools. It is a symptom of gastrointestinal infection caused by a virus, bacteria or parasite and can be acute (short term) or chronic (long term) lasting more than two to three weeks

Diarrhoea occurs worldwide and most people are affected by diarrhoea at some time in their lives. Globally, 2.2 million people die every year from diarrhoeal diseases (including cholera); 90% are children under 5 years(1) mostly in developing countries and these children die from complications of diarrhoea such as dehydration and malnutrition.

The inclusion of water supply, sanitation and hygiene in the millennium development goals underscores the fact that the world community has acknowledged the importance of their promotion as timely interventions to curtail under 5 mortality resulting from diarrhoea-related cases.

In Ghana today, under 5 mortality rate though has improved significantly from a high 215 in 1960 to 111 in 2003(2), a lot of concerns have been raised especially considering the fact that there has been an apparent slowing down in the mortality decline from 1989-2003, a decline of only 119 to 111(2). Hence, the Ghana Poverty Reduction Strategy target of achieving an infant mortality rate of ninety five per thousand (95/1000) by 2005 is under threat(1). Mortalities resulting from diarrhoea is one of the main factors mitigating against the attainment of these goals.

## METHODOLOGY

Koforidua is one of the four sub municipals in the New Juaben District in the Eastern Region of Ghana. It is the capital of the Eastern Region and it lies between latitude 60 N and 70 N.

The study was a case control study which was conducted between 17<sup>th</sup> April and 19<sup>th</sup> May, 2006 within the Koforidua municipality.

The study population was made up of two groups of fifty (50) each. The first group (CASE GROUP) was made up of parents who had children less than five (5) years old with diarrhoea. The second group was made up of parents with children less than five years old who did not have diarrhoea but were reporting with different sicknesses (CONTROL GROUP).

Both groups were interviewed at the child health out-patient department of the Koforidua Regional Hospital. Records of the hospital were also reviewed for diarrhoea cases in the previous two years. Systematic random sampling and Convenience sampling methods were used. For the control group, every third (3<sup>rd</sup>) parent was interviewed and for the case group, every parent was interviewed. The sample size was also selected by convenience.

The questionnaires were administered to respondents after explaining to them the

purpose of the study and assurance of strict confidentiality.

Responses were recorded immediately on the questionnaire.

The data was analyzed manually using Microsoft Office Excel. Participant's responses were converted into frequencies and percentages. The results were further represented on tables and charts.

Odds Ratio (OR) estimation and chi square analysis were done to establish the association between the various sanitation and hygiene practices and incidence of diarrhoea.

## RESULTS

Table 1 shows the Demographic characteristics of the Respondents. Of the two groups used for the survey, children of the Respondents aged between 24 and 59 months were in the majority (42% for the case group, 54% for the control group).

Table2 summarizes the factors influencing the incidence of diarrhoea in Koforidua.

All 100 mothers interviewed had heard about diarrhoea. Majority of the mothers mentioned Ante-natal clinic (ANC) as the source of their knowledge (58% of the case group, 64% of the control group). The rest of the mothers mentioned other media such as TV/Radio, friends, home and school as their sources of information. As shown in Figure 1 the prevalence of diarrhoea in Koforidua has generally been increasing from 4143 in 2002 to 6127 in 2005.

De demons d	C (NI-E0)		
Background	Cases (N=50)	Control (N=50)	
Characteristic	%	%	
CHILD'S AGE(months)			
0-11			
12-23	38	36	
24-59	42	54	
SEX OF CHILD			
Male	64	44	
Female	36	56	
MOTHER'S MADITAL STATIS	2		
MOTHER 5 MARITAL STATUS			
Married	74	66	
Single	26	30	
Divorced	0	4	

Table 1 Background Characteristics of Respondents

MOTHER'S RELIGION			
Christian	96	84	
Moslem	4	12	
Traditional	0	4	
MOTHER'S EDUCATION			
None	4	18	
n i	1	10	
Primary	16	6	
Secondary	64	68	
Tertiary	22	8	
MOTHER'S OCCUPATION			
Trader	60	70	
Artisan	22	2	
Farmer	0	6	
Formal	8	0	
Unemployed	10	12	

# TABLE 2: Summary of results on factors influencing the incidence of diarrhoea in Koforidua

Municipality, Ghana

		95% Confidence	
Risk factor	Odds ratio	Intervals	
Knowledge			
Poor knowledge on causes of diarrhoea	7.53	3.23 - 17.58	
Poor knowledge on prevention of	4.55	1.89 - 10.99	
Diarrhoea			
Personal Hygiene			
No hand washing before preparation of	1.09	0.47 - 2.54	
Meals			
No hand washing before feeding a child	0.52	0.23 - 1.16	
No hand washing after using the lavator	y <b>10.44</b>	3.34 -32.56	
Food and Water Safety			
Child less than six months not being	21.00	7.93 - 55.63	
Exclusively breastfed			
Fruits not washed before being eaten	0.64	0.40 - 1.00	
Water reservoirs not covered	10.10	4.20 - 24.50	
Domestic waste/Excreta management			
Waste not covered at home	4.51	2.07 - 9.83	
Unsafe mode of domestic waste disposal	3.14	1.13 - 8.70	
(not buried)			
Unimproved toilet facilities	17.47	6.61 - 46.10	
Treatment of diarrhoea			
Rehydration not a first line option	11.22	4.7 - 26.80	

#### P<0.05

#### Figure 1 : PREVALENCE OF DIARRHOEA IN KOFORIDUA (2002-2005)



#### SOCIODEMOGRAPHIC INFORMATION

According to the Ghana Demographic Health Survey (GDHS) 2003, children less than six months are less likely to have diarrhoea due to their being exclusively breastfed and hence their lower level of exposure to contaminated food or water<sup>2</sup>. However, this was not seen in this analysis and the reason could possibly be due to the fact that the category for exclusively breastfed infants extended beyond 6months to 10months, by which time the children must have started receiving supplementary feeds.

It is observed that more males (64%) had diarrhoea in the case group as against 36% of females. In the control group, 44% of the children were males whilst 56% were females. This result is possibly due to the unequal distribution of males and females in both groups

Majority of the respondents in both groups were married, 74% and 66% for the case and control groups respectively. This suggests that most children were born into stable family homes. The results further show that 96% and 84% of the case, and control groups respectively were Christians, 4% and 12% of the case and control groups respectively were Moslems whilst 4% of the control group were traditionalists. This is a true reflection of the population in Koforidua which is predominantly Christian.

The mothers interviewed were generally well educated with 60% to 80% of the entire population having pursued at least secondary

education. Interestingly, 60% of mothers in the case group were in this category. This finding does not agree with the observation made in the GDHS 2003 that the higher the level of education of a mother, the less likely it is for her child to get diarrhoea (3). This probably could be explained as being due to a gap existing between knowledge acquired and its application. Oni (1996) however recorded a similar finding in his study done in Ilorin, in Nigeria (3). Children of mothers with secondary education had significantly higher risk of diarrhoea compared with children of illiterates (OR = 1.9; P < 0.05).

Most of the mothers were gainfully employed and earned modest income and majority of them were traders, 60% and 70% of the case and control groups respectively. 10% and 12% of the case and control groups respectively were unemployed.

#### KNOWLEDGE ABOUT DIARRHOEA

As shown in Table 2, the fact that most of the mothers heard about diarrhoea at ANC is perhaps an indication that there is increased patronage of ante-natal clinic services. This correlates with the data in the GDHS 2003 which indicates that there has been an 11% increase in utilisation of ANC services in the past fifteen years (3).

To ascertain whether mothers had detailed knowledge about diarrhoea, further questions were asked. This revealed that only 32% of the case group identified contaminated water or food as the cause of diarrhoea as against 78% of the control group which regarded contaminated food or water as causing diarrhoea. Besides, as much as 56% of the case group did not know the causes of diarrhoea as against 18% of the control group.

In addition, 82% of the control group answered 'yes' to the question 'can diarrhoea be prevented?', only 50% of the case group answered in the affirmative to the question. Added to these findings was the fact that only 36% of the case group regarded the practising of good hygiene as a way of curbing diarrhoea whilst 70% of the control group expressed likewise opinion. Indeed the study revealed that the depth of knowledge about diarrhoea in the case group was very shallow and inadequate and this may be due to the lack of understanding of the information they gained from the various sources. Indeed this analysis revealed that poor knowledge about the cause of diarrhoea increased the odds of reporting a case of diarrhoea by a factor of 7.53.

#### PERSONAL HYGIENE

Poor personal hygiene appears to be related to a high incidence of diarrhoea (Table 2). Only 40% of the case group practised hand washing with soap and water before preparation of meals and 42% of the control group also engaged in this practice. Further, that just 60% of the case group practised hand washing with soap and water after using the lavatory, whilst 94% of the control group practised this all the time. This is possibly a reflection on the lack of in depth knowledge about the transmissible routes of diarrhoea especially within the case group. The study thus indicated that the incidence of diarrhoea was greater in groups with poor practice of hand washing prior to the preparation of meals and after using the lavatory, with estimated odds ratio value for the former being 1.09.The chi square test of association between poor hand washing practise after using the lavatory was of the value 16.32 which at p value of 0.05 is statistically significant at one degree of freedom.

In many ways this analysis confirms results from several other studies such as one done by Huttly et al which revealed that poor personal hygiene leads to increased prevalence of diarrhoea(4). Relationship between hygiene practices and infantile health has also been identified in several investigations like those developed in Bangladesh and Ashworth et al also recorded an 11% reduction in the incidence of diarrhoeal diseases in some communities in Zaire where personal hygienic practices were improved(5).

#### FOOD AND WATER SAFETY

Very few mothers with children less than six months in the case group (12.5%) practised exclusive breastfeeding as compared to 75% of mothers of the control group. Breast milk is known to be protective against various gastrointestinal infections due to the presence of lactoferrins and lysozyme in the breast milk. These enzymes are known to have bactericidal, fungicidal and virucidal activity and the absence of this protective function in non-exclusively breastfed infants possibly accounts for the increased incidence of diarrhoea in this group.

Besides, while 56% of the case group use water from wells for their domestic activities, majority (70%) of the control group use pipe-borne water. Most of the mothers interviewed revealed that the wells from which they fetched water were unprotected and the wells were also subject to seasonal fluctuations in the water level which led to discolouration(and associated contamination) of the water especially in dry season. Also, 24% of mothers in the case group do not cover water which is stored for use in the future as against 76% of mothers in the control group who keep their stored water covered. These modes of contamination must have led to the increased incidence of diarrhoea in the case group. Water reservoirs which were not covered appeared to be significantly associated with the incidence of diarrhoea with an estimated odds ratio value of 10.08 (Table 2).

When odds ratio and chi square test was performed on the data, whether or not fruits were washed before being eaten did not emerge as a significant determinant of prevalence. diarrhoea This might presumably be because of the small sample sizes; only more pronounced hygiene behaviours were statistically significant on their own. If one however looks at the overall picture, it is striking how consistent the trend is of increasing incidence of diarrhoea with poorer hygiene behaviour.

In many ways this analysis confirms results obtained from several other studies such as one carried out in East Africa (Uganda, Tanzania and Kenya) which reported that use of surface water showed significant odds ratio of 1.75(6). This was broadly consistent with earlier findings made which reported a significant association between diarrhoea prevalence and drinking surface water.

### DOMESTIC WASTE MANAGEMENT AND HUMAN EXCRETA DISPOSAL

As in other studies, there is a very high association between poor waste management and excreta disposal and incidence of diarrhoea. Results from this study show that majority of mothers in the case group (78%) kept domestic waste uncovered and 36% of mothers in the control group also did likewise. Keeping domestic waste uncovered increased the odds of having diarrhoea by 4.51 times. There were also poor waste disposal methods in both groups but this is worse in the case group, with 88% of mothers disposing their waste in the community dumping site (Exposed) as against 70% by the control group (Table 2).

In addition to the poor waste disposal methods seen in the case group, they also have the challenge of poor human excreta disposal, with only 10% of the group having access to flushing water closets and majority of the group disposing off their waste in pit latrines and the bush. In the control group, as many as 70% of the population have access to flushing water closets with just 10% resorting to disposal of excreta in the bush. The use of unimproved toilet facilities was significantly associated with the incidence of diarrhoea and an odds ratio of 17.47 was obtained.

An inadequate management of domestic waste refuse showed an odds ratio of 2.48 for infantile diarrhoea in Nigeria<sup>3</sup> and similar result was also observed in Brazil.

#### TREATMENT OF DIARRHOEA

From this study, 52% of mothers whose children had diarrhoea said that their first intervention during an episode of diarrhoea will be to send the sick child to hospital. 86% of the control group also had this option as their topmost priority. However, 42% of the case group admitted to the practise of self-medication and out of this proportion, only 22% of them thought of rehydrating their children with oral rehydration salt (ORS). For the control group, 14% practised selfmedication and out of this proportion 76% provide rehydration through the use of ORS. Poor practice of rehydration was found to be significantly associated with diarrhoea. A test using chi square analysis gave a value of 29.17 which is statistically significant at a p value of 0.05 at on degree of freedom (Table 2).

67% of the children with diarrhoea experienced recurrence within one month

after the first episode whilst 21% of the control group also experienced recurrence.

The concept of oral rehydration therapy (ORT) rarely occurs to mothers when their children get diarrhoea and this assertion is strengthened by the results of this study especially among the case group. Poor or inappropriate treatment offered children suffering from acute episodes of diarrhoea lead to recurrence and this can be complicated by dehydration which plays a vital role in the upsurge of morbidity resulting from diarrhoeal illnesses.

Most of the mothers interviewed admitted to the fact that accessibility to healthcare is not a problem (94% for case group, 90% for control group) since most of them were registered on the National Health Insurance Scheme (NHIS).

# PREVALENCE OF DIARRHOEA IN KOFORIDUA

Key informant interviews with some health workers attributed the high prevalence to the improved timeliness of weekly reporting of communicable diseases by the disease control and prevention unit of the public health department (Figure 1).

Further the easy accessibility to healthcare that has been made possible by the recently introduced National Health Insurance Scheme (NHIS) was also said to have contributed to the increased number of diarrhoea cases reported to the health facilities.

Direct interaction with the inhabitants also revealed that the supply of pipeborne water is very irregular and hence many homes depend mainly on surface water, notably wells, for water for domestic purposes.

It was also observed from this study that a far greater proportion of the diarrhoea population consistently failed to practise the good principles of hygiene such as hand washing before meals. Many of such mothers who engaged in some of the 'good practices' did it just due to 'basic reasoning' and not out of understanding for the mode of transmission of the disease.

#### CONCLUSION

The study showed that there has been an increase in the prevalence of diarrhoea in the Koforidua municipal in the last three years and this is a health challenge.

All mothers had heard about diarrhoea and most mothers acquired their knowledge about diarrhoea from Ante-Natal Clinic.

The depth of knowledge of the case group was poor relative to the control group; fundamental knowledge about the cause and mode of prevention of diarrhoea was largely not known to this group. incidence of diarrhoea in this group appeared to be associated with the poor knowledge about the disease.

Personal hygiene was a real challenge in the case group. A significant association between poor hygiene and occurrence of diarrhoea was established from this study.

## RECOMMENDATIONS

#### Government and policy makers

The government should be more proactive in the provision of good infrastructure for disposal of refuse. Waste reservoirs at community dumping sites should be emptied regularly to prevent overflow of refuse. Proper legislature should be made on the provision of standard toilet facilities in every home and water should be made available within reasonable distance from homes. The pipe-borne water supply to the township should be expanded to ensure wider coverage and regular delivery of safe water to homes.

The work of sanitary inspectors in the community should be enforced and properly supervised to ensure good standards of sanitation in the communities. Oral rehydration therapy (ORT) and good hygiene techniques should be taught at all stages of education. The Ministry of Health (MOH) and Ministry of Education (MOE) should be responsible for incorporation of this into school syllabus.

## **Healthcare Providers**

The role of healthcare providers in the reduction of infant mortality resulting from diarrhoeal illness has been proven to be invaluable by this study. More women are accessing ANC services and health personnel should thus ensure that education on management and prevention at this level is heightened. The message should be simple, and delivered in unambiguous language to eliminate the communication gap. Demonstration with visual aids will enhance the understanding of the fundamentals of the disease.

There is also the need for healthcare providers to be courteous and affable to patients so that their services will be patronized readily.

#### **Role of Community and Individuals**

The local radio stations should be encouraged to bring on health experts to discuss diarrhoea on their networks since quite a significant number of people enjoy their programmes. Mobile health vans should be used to propagate information on diarrhoea in various communities. Women in the main market should be a major target in this direction.

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